

## **Comparison between two multivariate analyses for the evaluation of genetic divergence for carcass and meat quality traits in alternative lines of chicken**

**Comparação entre dois métodos de análises multivariadas para avaliação de divergência genética para características de carcaça e qualidade de carne em linhas de frango colonial**

**Comparación entre dos metodos de análisis multivariado para evaluar la diferencia genética para los rasgos de la canal e la calidad de carne em líneas alternativas de pollo**

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**Túlio José de Freitas Goes**

ORCID: <https://orcid.org/0000-0001-7753-2580>  
Universidade Federal Fluminense, Brazil  
E-mail: [tulio Goes\\_6@hotmail.com](mailto:tulio Goes_6@hotmail.com)

**Cláudia del Castilho**

ORCID: <https://orcid.org/0000-0003-2046-0234>  
Universidade Federal Fluminense, Brazil  
E-mail: [claudiacris82@yahoo.com.br](mailto:claudiacris82@yahoo.com.br)

**Rogério de Carvalho Veloso**

ORCID: <https://orcid.org/0000-0003-3244-7336>  
Instituto Federal Goiano, Brazil  
E-mail: [rogerio.veloso@ifgoiano.edu.br](mailto:rogerio.veloso@ifgoiano.edu.br)

**Gabriela Ramos Leal**

ORCID: <https://orcid.org/0000-0002-2167-8780>  
Universidade Federal Fluminense, Brazil  
E-mail: [gabivetlea@gmail.com](mailto:gabivetlea@gmail.com)

**Rodolpho de Almeida Torres Filho**

ORCID: <https://orcid.org/0000-0003-4047-4932>  
Universidade Federal Fluminense, Brazil  
E-mail: [ratfilho@id.uff.br](mailto:ratfilho@id.uff.br)

### **Abstract**

The univariate analysis is becoming harder to use due the increasing number of characteristics of economic importance in agricultural industry. The multivariate approach provides an answer to this issue, allowing to analyze several traits when comparing different methodologies, genetics and products. This study aimed to use and compare the canonical variable analysis (CVA) and principal component analysis (PCA) to evaluate 7 genotypes of alternative lines of chicken (Caboclo, Carijó, Colorpak, Gigante Negro, Pesadão Vermelho, Naked Neck and Tricolor). The study evaluated 840 male chicks reared at 91 days from these genotypes, in a completely randomized design with 4 replicates per genotype. Different traits (23) were measured, and only 7 remained relevant after the multivariate approach: Carcass yield, Breast Yield, Back Yield, Cooking Loss, lightness, yellowness and water holding capacity. Both analyses remained with two variables explaining the varying. The Pearson correlation was used to measure the traits responsible for the most variance between genotypes. On the principal component cooking loss, carcass and breast yields, and the color parameters lightness and yellowness were the most relevant, while on canonical variables it was carcass yield, breast yield, lightness, yellowness and back yield. Both analyses resulted in similar conclusion, allowing to classify the genotypes in three major groups: 1 (Pesadão vermelho, Carijó, Colorpak, Naked Neck), 2 (Gigante Negro and Caboclo) and 3 (Tricolor). PCA and CVA facilitate the interpretation of data with several traits of importance, showing the main traits responsible for genetic divergence.

**Keywords:** Principal component; Canonical variables; Meat trait; Quality traits.

### **Resumo**

A análise univariada está se tornando mais difícil de usar devido ao crescente número de características de importância econômica na indústria agrícola. A abordagem multivariada permite avaliar várias características na comparação de diferentes metodologias, genética e produtos. Este estudo utilizou e comparou a análise de variáveis canônicas (CVA) e a análise de componentes principais (PCA) para avaliar 7 genótipos de linhagens de frango colonial (Caboclo, Carijó, Colorpak, Gigante Negro, Pesadão Vermelho, Naked Neck e Tricolor). Avaliaram-se frangos machos (840) criados até os 91 dias, em um delineamento inteiramente casualizado com 4 repetições por genótipo. Diferentes características (23) foram mensuradas e apenas 7 permaneceram relevantes após abordagem multivariada: Rendimento de carcaça, peito, e dorso, perda por cocção, luminosidade (L\*), teor de amarelo (b\*) e

capacidade de retenção de água. Ambas as análises permaneceram com duas variáveis respondendo pela maior parte da variabilidade. A correlação de Person foi utilizada para identificar as características responsáveis pela maior variabilidade entre os genótipos. Na PCA, a perda por cocção, os rendimentos de carcaça e peito, e os parâmetros de cor ( $L^*$  e  $b^*$ ) foram os mais relevantes, enquanto na CVA foram rendimento de carcaça, peito e dorso, e os parâmetros de cor ( $L^*$  e  $b^*$ ). Ambas as análises resultaram em conclusões semelhantes, permitindo classificar os genótipos em três principais grupos: 1 (Pesadão vermelho, Carijó, Colorpak, Naked Neck), 2 (Gigante Negro e Caboclo) e 3 (Tricolor). PCA e CVA facilitaram a interpretação de dados com várias características de importância, mostrando as principais responsáveis pela divergência genética.

**Palavras-chave:** Componente principal; Variáveis canônicas; Características de carne; Características de qualidade.

### Resumen

El análisis univariado es cada vez más difícil de utilizar debido al creciente número de características de importancia en la industria agrícola. Un enfoque multivariado proporciona una evaluación de múltiples rasgos frente a diferentes metodologías, genéticas y productos. Este estudio utilizó y comparó el análisis de variables canónicas (CVA) y el análisis de componentes principales (PCA) para evaluar 7 genotipos en líneas alternativas de pollo (Caboclo, Carijó, Colorpak, Gigante Negro, Pesadão Vermelho, Naked Neck and Tricolor). Se evaluaron 840 pollos machos criados hasta 91 días, en diseño completamente aleatorizado con 4 repeticiones por genotipo. Se midieron diferentes características (23) y solo quedaron 7 después del enfoque multivariado: rendimiento en canal, pechuga, y dorso, pérdida de cocción, luminosidad ( $L^*$ ), contenido de amarillo ( $b^*$ ) y capacidad de retención. Ambos análisis se mantuvieron con dos variables que explican la mayor parte de la variabilidad. Se utilizó la correlación de Pearson para identificar características responsables por la mayor variabilidad entre genotipos. En PCA, las pérdidas por cocción, los ajustes de canal y pechuga, y los parámetros de color ( $L^*$   $b^*$ ) fueron los más relevantes, mientras que en el CVA fueron rendimiento en canal, pechuga y dorso, y los parámetros de color ( $L^*$   $b^*$ ). Los análisis fueron similares y permitieron clasificar los genotipos en tres grandes grupos: 1 (Pesadão Vermelho, Carijó, Colorpak, Naked Neck), 2 (Gigante Negro y Caboclo) y 3 (Tricolor). PCA y CVA facilitaron la interpretación de datos con varias características importantes, mostrando las principales responsables por la divergencia genética.

**Palabras clave:** Componente principal; Variables canónicas; Características de la carne; Características de calidad.

## 1. Introduction

In tropical and sub-tropical regions, the heat stress is a significant challenge on broiler production. Since the largest region that produces broilers are tropical regions, this matter is an important one to overcome, leading to losses of growth rate, feed intake, feed efficiency, hatchability and increasing the mortality rate. One of the solutions is to use the beneficial adaptability of colored chicken, like the ‘naked neck’ genetic line. This is just one example of chickens that have a higher capacity of heat dissipation, withstanding increased heat stress (Rajkumar et al., 2011).

There are several traits related to quality on poultry meat, therefore becoming a complex issue to evaluate. Yields, scores, sensory and nutritional parameters, all must be evaluated to attract the consumer and having a better product. (Bayyurt et al., 2018).

When discussing the complexity of the genetic relationship between the different traits, it is stated that the use of univariate analysis to interpret differences neglects the existence of correlations between them. However, the multivariate methodology can consider the nature of the relation between them, being more appropriate to be used when a high number of traits need to be evaluated. Among the multivariate analysis techniques, the technique of principal components analysis is highlighted, originally described by Pearson (1901). This method consists in transforming the original set of variables into another: the principal component (PC). The PC are of equivalent dimensions, but with the property that each component retains a percentage of the original total variance and that the variances decrease from the first to the last component (Udeh & Ogbu, 2011).

The canonical variable analysis (CVA) also allows to analyze a set of variables, being important to verify the genotype divergence. The canonical variable (CV) use uni or multidimensional dispersion diagrams to verify the scores of similarity. The CVA transform a set of original variables into standardized non correlated variables, using the mahalanobis  $D^2$  distance to keep the conglomeration process, and also making use of covariance and residual matrices of the original variables

evaluated (Veloso et al., 2016).

The CVA goal is to maximize the differences among groups and minimize within groups thus becoming easier to visualize which traits causes the most genetic divergence and the relation between genotypes and which traits are the most significant in those differences (Ogah, 2013).

It is important to note that the PCA and CVA are the main techniques of multivariate analysis in use on several studies in animal production. However, most researchers still use univariate analysis, a limited approach when the need to evaluate several variables exist (Traldi et al., 2018). The present study aimed to evaluate the results of both techniques, PCA and CVA, and their differences in evaluating the genetic divergence between 7 genotypes of colored chicken for meat yield and meat quality characteristics.

## 2. Methodology

This experiment was approved by the Ethics Commission for Animal Use of the Universidade Federal dos Vales dos Jequitinhonha e Mucuri (protocol number 015/10).

**Study Location:** The database used in this study was originated from the experiment conducted in the poultry sector of the department of animal husbandry (DZO) of the Universidade Federal dos Vales dos Jequitinhonha e Mucuri (UFVJM) in Diamantina-MG from February 21 to May 16, 2011 and approved by the Ethics Commission for Animal Use.

**Experimental Animals and their management:** A total of 840 male chicks were collected from seven broiler type genotypes of the redbro lineage: Caboclo (CBC), Carijó (CG), Colorpak (CPK), Gigante Negro (GNG), Pesadão Vermelho (PS), Pescoço Pelado or Naked Neck (PP) and Tricolor (TRC). The experimental design was completely randomized, with 4 replicates per genotype. Each experimental box had 2.00 x 2.00m, with 30 birds per box, in masonry shed, covered by asbestos cement tiles. The breeding system was semi-intensive, and from the 28 days of age, the birds had access to a 30m<sup>2</sup> picket, formed by Tifton-type forage, Cynodon. The rations were formulated with corn and soybean meal for the initial (1 to 28 days), growth (28 to 56 days) and final (56 to 91 days) phases. At 91 days of age, after an 8 hour fast, two animals were randomly withdrawn from each box and sent to the slaughter room of the DZO / UFVJM. After slaughter and all evisceration process, the carcasses were weighed still warm and the abdominal fat was removed.

**Traits Measured:** The carcass yield was obtained by the relation between the weight of the cold carcass (without feet, head and neck) and fasting weight. The yield of breast (BRE), thigh (THG), drumstick (DRU), wing (WIN) and back (BAK) were obtained by the relation between the weight of these parts and the weight of the cold carcass. The proportions of feet (FEE), abdominal fat (AFA), total viscera (VIS), liver (LIV), gizzard (GIZ) and heart (HRT) were also obtained. Color (L \*, a \*, b \*), pH, texture (TX), moisture (UM), water holding capacity (WHC) and cooking loss (CL) were measured in the science and technology sector of animal products of DZO. The analyses of protein (PTN%), lipids (EE%) and ash (CZ%) were carried out in the animal nutrition laboratory of the Universidade Federal Fluminense.

**Statistical Analyses:** The Shapiro-Wilkov test was performed at 5% significance, while the homogeneity of variance was determined through the Bartlett test. One-way analysis of variance was used to test the effect of the genetic line of broiler on the parameters. Two methodologies were used in the multivariate analysis: Principal Component Analysis (PCA) and Canonical Variable Analysis (CVA).

The Principal Component Analysis (PCA) consist in explaining the variance and covariance of a randomized vector made of “p” random variables using non-linear combinations on the original variables. Those combinations are called Principal Components. The principal components are obtained by transforming variables in a multivariate data set ‘x1, x2, ... xp’ into new uncorrelated variables ‘y1, y2...yp’ which account for decreasing proportions of the total variance in the original variables defined as:

$$y_1 = a_{11}x_1 + a_{12}x_2 + \dots + a_{1p}x_p.$$

$$y_2 = a_{21}x_1 + a_{22}x_2 + \dots + a_{2p}x_p.$$

$$y_p = a_{p1}x_1 + a_{p2}x_2 + \dots + a_{pp}x_p.$$

The principal components 'y<sub>1</sub>, y<sub>2</sub>...y<sub>p</sub>' account for decreasing proportions of the total variance in the original variables 'x<sub>1</sub>, x<sub>2</sub>...x<sub>p</sub>'.

The Kaiser criterion (Kaiser, 1960) was used to select the least relevant PC. This criterion takes into account only those principal components with eigenvalues above the unit. The eigenvalue of a principal component is associated with the variance of all traits included in the principal component. As described by Jolliffe (1973), each variable was associated with each of the PC's, and those variables associated with the components of eigenvalue below one, were discarded. It was selected the PC's that explain most of the genetic variation of the data, where it maintains accumulate approximately 80% of the total variance.

The canonical variables analysis (CVA) was performed using the same variables that were selected in the PCA. After excluding the variables with the least impact in the total variance, it was used mahalanobis distance to verify the different groups and to obtain the canonical variables (CV).

Data was tabulated using spreadsheets from the Microsoft Office Excell program. The univariate and multivariate analyses were performed through the PROC GLM procedure in the SAS software, whereas the PCA and the correlations used the procedures PROC PRINCOMP and PROC CORR, respectively, in this same software. The CVA was performed using the PROC CANDISC, while the difference between groups used the procedure PROC GLM.

It was used the microsoft Office Excel program to create the bi-plot figure using the PC and CV selected as described by Hongyu et al.(2015).

### 3. Results

The verification of normality tests and homogeneity of variances indicated that there was no need for data transformation. The results of the univariate analysis are described in Table 1.

**Table 1.** Means for carcass traits evaluated in different genetic lines of chicken.

TRAITS	PP	OS	TRC	CG	CPK	CBC	GNG
<b>LW*</b>	3.91 c	4.32 b	4.21 bc	4.48 ab	4.73 a	3.08 d	3.37 d
<b>CAR*</b>	69.66 ab	70.23 a	68.05 abc	69.53 ab	70.22 a	65.05 c	66.45 bc
<b>BRE*</b>	32.46 ab	32.76 a	30.72 abc	32.84 a	32.40 ab	28.36 c	29.68 bc
<b>DRU*</b>	16.76 abc	16.55 bc	17.66 ab	17.01 abc	16.10 c	18.04 a	17.78 ab
<b>THG</b>	18.99	17.93	18.15	18.23	17.98	18.97	18.19
<b>WIN*</b>	11.99 abc	11.90 bc	12.07 abc	11.27 c	11.17 c	12.96 a	12.81 ab
<b>BAK</b>	20.42	20.91	21.62	20.83	22.51	22.02	21.82
<b>VIS*</b>	11.01 ab	9.62 b	10.69 ab	10.42 ab	9.72 b	11.68 ab	12.22 a
<b>HRT</b>	0.5	0.52	0.54	0.48	0.55	0.52	0.48
<b>LIV*</b>	2.09 a	1.58 b	1.57 b	1.67 b	1.51 b	1.73 ab	1.79 ab
<b>GIZ*</b>	2.11 bc	1.89 c	2.48 bc	2.48 bc	1.71 c	3.39 a	2.79 ab
<b>AFA*</b>	1.89 a	1.57 ab	1.71 ab	1.65 ab	1.79 a	0.92 b	0.92 b
<b>FEE*</b>	3.63 bc	3.57 bc	3.78 bc	3.59 bc	3.42 c	4.25 a	3.91 ab
<b>LP*</b>	0.82 abc	1.28 a	1.02 abc	1.42 a	1.12 abc	0.50 c	0.67 bc
<b>PT</b>	24.58	24.18	24.77	24.67	24.83	25.27	24.28
<b>AS</b>	1.11	1.13	1.15	1.12	1.13	1.07	1.11
<b>MS</b>	74.46	73.35	72.87	73.35	73.56	74.05	73.51
<b>CL</b>	20.68	24.05	25.01	20.45	23.28	22.12	18.88
<b>TX</b>	1.83	1.96	2.17	1.94	1.88	2.39	2.04
<b>L*</b>	55.68 ab	54.99 ab	57.21 a	53.41 b	54.93 ab	57.30 a	57.74 a
<b>A</b>	4.28	3.83	2.70	3.16	3.41	2.43	2.67
<b>B</b>	9.3	7.95	9.47	8.26	8.43	10.79	8.03
<b>pH*</b>	5.70 ab	5.69 ab	5.65 ab	5.75 a	5.71 ab	5.64 b	5.65 ab
<b>WHC</b>	57.10	56.82	55.93	55.33	58.25	56.44	57.34

Means with different letter in the row are significative different ( $p>0.05$ ); traits with \* had significative effect in the univariate analysis of variance.

Means of Live Weight (LW), Carcass Yield(CAR), breast yield (BRE), drumstick yield (DRU), thigh yield (THG), wing yield (WIN), back yield (BAK), viscera yield (VIS), heart yield (HRT), liver yield (LIV), gizzard yield (GIZ), abdominal fat yield (AFA), Feet Yield (FEE), lipids(LP), Protein(PT), ash (AS), moisture (MO), cooking Loss(CL), texture(TX), lighness(L\*), redness(a\*), yellowness(b\*), pH(PH) and water holding capacity(WHC) for each genotype: Pescoço Pelado (PP), Pesadão Vermelho (PS), Tricolor(TRC), Carijó(CG), Colorpack(CPK), Caboclo (CBC) e Gigante Negro (GNG).

Source: means from univariate analysis from owned data

Table 1 shows a database with several traits that be significant in the genetic divergence. Live Weight, Carcass Yield, Breast Yield, Dumstick Yield, Wind yield, Viscera Yield, Liver Yield, Abdominal Fat Yield, Feet Yield, lipids, L\* and pH were all significant. This table also shows which means were significative different between genotypes on each trait analysed.

Using the PCA, from the 23 initial variables, 7 explained most of the variance, indicating to be the most relevant to be used in the multivariate analysis. The characteristics that remained in the multivariate analysis were Carcass yield (CAR), Breast Yield (BRE), Back Yield (BAK), Cooking Loss (CL), L\* (L), yellowness b\* (B) and water holding capacity (WHC). The following variables were discarded through CP analysis: pH(PH), heart yield (HRT), ash(AS), live weight (LW), liver yield (LW), texture (TX), feet yield (FEE), lipids (LP), gizzard yield (GIZ), protein (PT), abdominal fat yield(AFA), wing yield(WIN), drumstick yield(DRU), viscera yield(VIS), redness a\*(A) and thigh yield (THG).

Table 2 shows how many principal components were needed to explain the total variance and how much variance each component responded in the principal component analysis (PCA). The same was described in table 3, but for the canonical variables in the Canonical Variable Analysis (CVA). The PC, their eigenvalues, the proportion of variance explained by each component, and the cumulative total variance are described in Table 2, while the same description by CVA are in Table 3.

**Table 2.** Eigenvalues, proportion of total variance and accumulated proportion per principal components (PC) selected from multivariate analysis.

<b>PC</b>	<b>Eigenvalue</b>	<b>Proportion of Total Variance</b>	<b>Accumulated Proportion</b>
<b>1</b>	22.066	50.83%	50.83%
<b>2</b>	10.034	23.11%	73.94%
<b>3</b>	5.401	12.44%	86.38%
<b>4</b>	2.369	5.46%	91.83%
<b>5</b>	1.813	4.18%	96.01%
<b>6</b>	1.253	2.89%	98.90%

Source: Correlations obtained from PROCCORR on SAS from owned data.

**Table 3.** Eigenvalues, proportion of total variance and accumulated proportion per canonical variables (CV) used in the multivariate analysis.

<b>CV</b>	<b>Eigenvalue</b>	<b>Proportion of Total Variance</b>	<b>Accumulated Proportion</b>
<b>1</b>	5,5582	72,68%	72,68%
<b>2</b>	0,9377	12,26%	84,94%
<b>3</b>	0,6005	7,85%	92,79%
<b>4</b>	0,3469	4,54%	97,33%
<b>5</b>	0,1731	2,26%	99,59%
<b>6</b>	0,0314	0,41%	100,00%

Source: Correlations obtained from PROCCORR on SAS from owned data.

By using tables 2 and 3, it can be seen that both PCA and CVA explained most of the variance by 6 principal components (PC) or canonical variables (CV). The only difference in the total of components or variables used was that while PCA explained 98.90% of the total variance using 6 components, CVA explained 100% total variance with 6 variables. When analysing the PC and CV needed to evaluate the difference between genotypes and most relevant characteristics in those differences, both PCA and CVA needed two vectors. However, while on the PCA two PC corresponded for over 70% of the total variance, CVA needed only one to achieve the same.

The results described in Table 4 and 5 demonstrate the Pearson correlation between the first two PC (Table 4) or CV (Table 5).

**Table 4.** Pearson Correlation among remaining traits and the first two principal components (PC).

<b>PC</b>	<b>Carcass Yield</b>	<b>Breast Yield</b>	<b>Back Yield</b>	<b>Cooking Loss</b>	<b>L*</b>	<b>b*</b>	<b>WHC</b>
<b>PC1</b>	0.20131	0.0599	0.15718	0.99674	-0.10896	0.09158	0.20422
<b>p-value</b>	0.3043	0.7621	0.4244	<0.0001	0.581	0.643	0.2972
<b>PC2</b>	0.86478	0.90637	-0.32521	-0.05964	-0.77739	-0.41469	0.02406
<b>p-value</b>	<0.0001	<0.0001	0.0913	0.7631	<0.0001	0.0282	0.9033

Source: Correlations obtained from PROCORR on SAS from owned data.

**Table 5.** Pearson Correlation among remaining traits and the first two canonical variables(CV).

<b>CV</b>	<b>Carcass Yield</b>	<b>Breast Yield</b>	<b>Back Yield</b>	<b>Cooking Loss</b>	<b>L*</b>	<b>b*</b>	<b>WHC</b>
<b>CV1</b>	0.89138	0.91193	-0.35250	0.08453	-0.72764	-0.40880	0.00985
<b>p-value</b>	<0.0001	<0.0001	0.0658	0.6689	<0.0001	0.0308	0.9603
<b>CV2</b>	0.04717	0.00661	0.61268	-0.12527	-0.23265	-0.31846	0.29363
<b>p-value</b>	0.8116	0.9734	0.0005	0.5253	0.2335	0.0986	0.1294

Source: Correlations obtained from PROCORR on SAS from owned data.

These results indicate that in the first PC, only the variable cooking loss was significant, demonstrating that for this component, this is the most relevant variable and cause of divergence among the genotypes. However, in the first CV, the variables Carcass Yield, Breast Yield, L\* and b\* were the most influential. The variables in the first CV are the same variables that had significant correlations in the second PC. In both the CV1 and PC2 the breast yield and the carcass yield have greater influence than the variables L\* and b\*, both which had negative correlations, indicating that the higher this CV or PC, the lower the values in those two variables. This indicates a divergent direction between carcass and breast yield, and L and b indexes. On the second CV, only back yield had a significant correlation

On tables 6 and 7 are described the mean scores of the first two PC (table 6) or CV (table 7) for each genotype.

**Table 6.** Scores for the first two Principal Components (PC) for each broiler genotype.

<b>GENOTYPE</b>	<b>PC1</b>	<b>PC2</b>
<b>TRC</b>	2.742	-1.628
<b>PS</b>	2.170	2.446
<b>CPK</b>	1.613	2.063
<b>CBC</b>	-0.362	-4.952
<b>PP</b>	-1.210	1.666
<b>CG</b>	-1.529	3.023
<b>GNG</b>	-3.425	-2.618

Broiler genotypes: Tricolor (TRC), Pesadão Vermelho (PS), Colorpack (CPK), Caboclo (CBC), Pescoço Pelado (PP), Carijó (CG) e Gigante Negro (GNG).

Source: means from PCA analysis from owned data.

**Table 7.** Scores for the first two Canonical Variables (CV) for each broiler genotype.

<b>GENOTYPE</b>	<b>CV1</b>	<b>CV2</b>
<b>TRC</b>	-0.696	-0.900
<b>PS</b>	1.985	-0.352
<b>CPK</b>	1.293	1.530
<b>CBC</b>	-3.665	-0.063
<b>PP</b>	1.283	-1.097
<b>CG</b>	1.898	0.279
<b>GNG</b>	-2.098	0.602

Broiler genotypes: Tricolor (TRC), Pesadão Vermelho (PS), Colorpack(CPK), Caboclo(CBC), Pescoço Pelado(PP), Carijó(CG) e Gigante Negro(GNG)  
Source: means from CVA analysis from owned data.

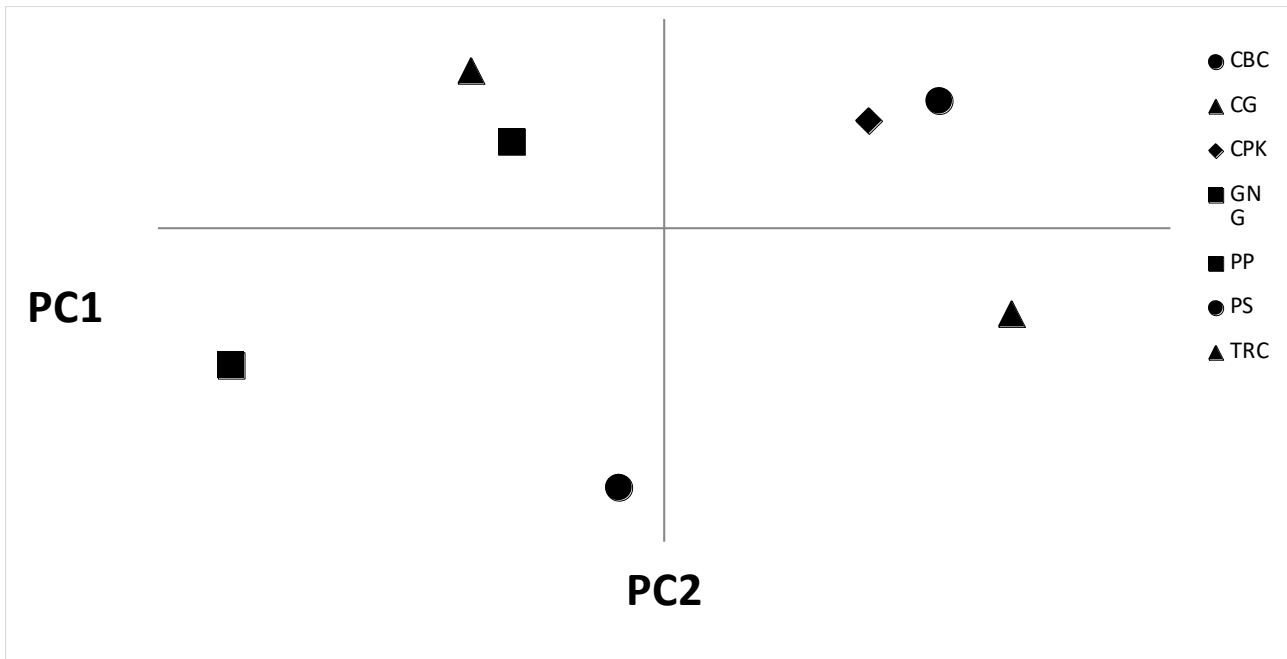
The Table 6 demonstrates that the genotypes TRC, PS and CPK had positive values for the means on principal component 1(PC1), and the genotypes CBC, PP, CG and GNG had negative means for the same component. However, on the principal component 2(PC2), the genotypes CG, PS, CPK and PP had positive and the highest means in order. And the genotypes which had negative means, by the lowest, were CBC, GNG and TRC.

In table 7, those results were not the same. For the canonical variable 1, the genotypes with positive means, by the highest, were PS, CG, CPK and PP, with genotypes CBC, GNG, and TRC having negative and the lowest means in order. However, in the canonical variable 2, the genotypes with negative values were PP, TRC, PS and CBC, and the genotypes CPK, GNG and CG having positive means.

These means in the tables 6 and 7 were used to create a scatter plot between the two PC (Figure 1) and between the first two CV (Figure 2) as Hongyu et al. (2015) described.

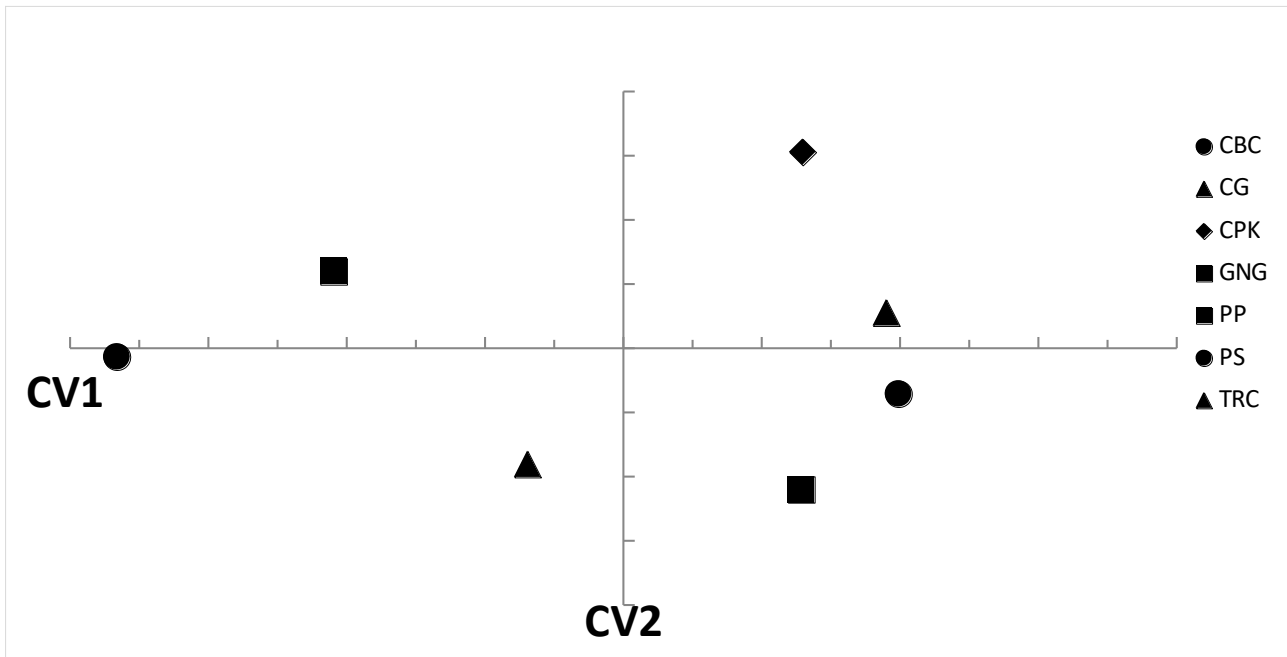


**Figure 1.** Scatter plot between principal component 1(PC1) and principal component 2(PC2) for the 7 genotypes evaluated: Caboclo (CBC), Carijo (CG), Colorpack (CPK), Gigante Negro(GNG), Pescoço Pelado (PP), Pesadão Vermelho (PS), Tricolor(TRC).



Source: Scatter plot made by Microsoft Office Excel from owned data.

**Figure 2.** Scatter plot between canonical variable 1(CV1) and canonical variable 2(CV2) for the 7 genotypes evaluated: Caboclo(CBC), Carijo(CG), Colorpack(CPK), Gigante Negro(GNG), Pescoço Pelado(PP), Pesadão Vermelho(PS), Tricolor(TRC).



Source: Scatter plot made by Microsoft Office Excel from owned data.

The scatter plot simplifies the evaluation of each genotype and the differences between them. Those results showed that in both analyses, the genotypes could be differentiated in four groups. In PCA (Figure 1), the CG and PP lines obtained

high PC2 scores but negative values for PC1. We can interpret that they were lines that presented high breast yield, associated to low L\* and b\*, and low rates of cooking loss. The CPK and PS lines had positive values for PC1 and PC2, indicating that they are lines with high breast yield, low scores of L\* and b\*, but a high level of cooking loss. The TRC line presented a high value for PC1, but PC2 negative, demonstrating that it was a line with a high score for cooking loss, low breast yield but with high L\* and b\*. The GNG strain presented negative values for both PC1 and PC2, indicating low cooking loss, low breast yield, and high L\* and b\*. And the CBC line, although also presenting PC1 and 2 negative, their values for CP1 presented values close to 0. This indicates that, despite having a cooking loss score higher than the 3 genotypes with PC1 positive, the CBC line presented lower values than the remaining 3 lines. However, this genotype had a low breast yield, and high L\* and b\*. While analyzing the scatter plot between CV (Figure 2), the lines CPK and CG had high CV1 and CV2, suggesting high values in carcass yield, breast yield and back yield, and low values of L\* and b\*. The lines PP and PS had high values for CV1, but negative values for CV2, corresponding to lines that have high carcass and breast yield but low L\*, b\* and back yield. The line GNG had high value for CV2 and negative value for CV1, indicating a genotype with low carcass and breast yield, but high values for colors (L\* and b\*) and back yield. And the lines TRC and CBC both had negative values for both CV1 and CV2. This indicates that these lines are genotypes with low carcass, breast and back yield, but high values for L\* and b\*.

Using both graphs we can conclude that we have 2 groups of genotypes. The ones with high carcass and breast yield, but low carcass color, composed of PP, PS, CG and CPK, with the differences between them being cooking loss or back yield. The other group are the ones with low carcass and breast yield, but high colorness of carcass, being composed of the three remaining lines, CBC, GNG and TRC. Both analysis indicate that the genotype CBC is the one with the lowest carcass and breast yield of the 7 genotypes.

#### 4. Discussion

The results presented in table 1 show that some characteristics have genotype effects, and also exist variation in the rank of the genotypes for each of them. Due to this, the interpretation of the results in the univariate analysis to compare 7 genotypes, considering a set of 23 variables, is too complex.

Venturini et al. (2013) selected 3 PC from a total of 19 original variables studied, accumulating 70.93% of the total variance. Those results were similar to the finding of Buzanskas et al. (2013), that also used two PCs, which accounted for 73.37% of the total variance. Ajayi et al. (2012) reduced 7 variables to 3 CP, accounting for approximately 80% of total variance in all the lines tested. Paiva et al. (2010) selected 3 PC accumulating 77% of the total variance.

Udeh and Ogbu (2011) used 7 variables for multivariate analysis, selecting 2 or 3 CP depending on the genetic line. In the Arbor Acre line, two PCs were selected, accounting for 64.97% of the total genetic variance. In their Marshal line, 3 PCs were selected, with total accumulated variance of 74.70. In the Ross line, which were also selected 3 CP, the total variance exceeded 80%.

Savegnago et al. (2011) also worked with 3 CPs, although 4 CPs met the Kaiser criterion, since they answered for 67.59% of the total variance. All of these studies present similar finds in total PC selected or total variance accumulated when compared with the PCA performed in this study. Paiva et al. (2010), Venturini et al. (2013), Buzanskas et al. (2013) and the Marshal Line of Udeh and Ogbu(2011), had similar total variance answered of the PC selected, but with only Buzanskas having the same number of PC, and the other needing one more PC. Ajayi et al. (2012) and the Ross Line of Udeh and Ogbu(2011) had higher total variance, but with one more PC required. And Savegnago et al. (2011) and the Arbor Acre line of Udeh and Ogbu(2011) had lower total variance compared to the PCA on the present study.

If these same studies are compared with the CVA performed in the current one, none of them presented less components or variables selected, with the same total variance analyzed.

Rosário et al. (2008) performed a CVA, acquiring two CV from a total of 6 variables, with a total variance of 96.49%. It had reduced 9 variables to 2 CV, with a total variance of 94.63%. Veloso et al. (2015a), Veloso et al. (2015b) and Veloso et al. (2015c), had two to three CV responding for 97.41%, 88,82% and 88,10% of total variance respectively. And Ozsoy (2019) had three CV responding for 77.51% of total variance. Only Ozsoy (2019) and Veloso et al. (2015b) had less total variance in two CV than the current study. If we use the PCA to compare the results showed by the authors, all of them presented less total variance responded than the PCA performed in this study.

With the comparisons between PCA and CVA analysis we can assume that the variables from CVA answer for more variance than the components of the PCA. So when using fewer total variables, the CVA can be an easier analysis to read the results.

Each study also specified how much of variance each PC or CV responded. Venturini et al. (2013) had 45.19% in PC1, 16.41% in PC2, and 9.33% in PC3. In Buzanskas et al. (2013), PC1 accounted for 48.51%, while PC2 accounted for 24.86% of this total. Each line of Ajayi et al. (2012) had different PC. The PC1, PC2, and PC3 were respectively 71.99%, 12.66% and 5.13% on the Normal Feathered line. Frizzle Feathered had 73.99%, 14.33% and 4.75% for each of the PC. The line Naked neck had for PC1 77.16%, for PC2 14.24% and for PC3 3.43%, while "Anak Titan" found for these same PC 52.79%, 16.08% and 12.17%. Udeh and Ogbu (2011), like Ajayi et al. (2012), also found different PC for each line. Arbor Acre had for PC1 40.58% and for PC2 24.38%, the Marshal line had: 38.24%, 22.13% and for PC3 14.39%, and the Ross line had 39.04%, 29.70% and 16.50%. Savegnago et al. (2011) found for PC1, PC2 and PC3 32.30%, 18.71%, 16.58% respectively.

The only studies that had for PC1 higher variance was Ajayi et al. (2012). However, all of the lines had less variance in PC2. For PC2 the only ones that had higher variance than this study were Buzanskas et al. (2013) and the lines Arbor Acre and Ross from Udeh and Ogbu (2011). However, those three results were similar to the variance of the PC2 of the current study. When comparing with the first CV, only Ajayi et al. (2012) had similar results, while all the other had less variance in the first component.

When analyzing CVA, Rosário et al. (2008) had in the first canonical variable 89.35% and 7.14% in the second. Veloso et al. (2015a) found for CV1 and CV2 69.30% and 25.33%. Veloso et al. (2015b) on the first three CV had 52.78%, 27.07% e 8.97%, while in another study, Veloso et al. (2015c) found 73.83% for CV1, and 14.27% for CV2. Ozsoy (2019) had 77.51% accounting for CV1.

Those results, similar to when comparing with the total PC or CV selected, or the total variance accounted, shows that the CVA always had the CV1 accounting for higher variance than the PC1. However, when comparing CV2 with PC2, only Veloso et al. (2015a and 2015b) had higher variance. When comparing the variances from CV1 found on those studies with the CV1 on the present study, only Rosário et al. (2008) showed a higher one.

Comparing the results from the current study with the others already described, we can assume that to account for a total variance higher than 70%, the PCA needed in average two components, with other needing at least three. However, in CVA, most of studies only needed one CV, with other needing two at max to reach the same 70% of variance. Since this study utilize the same variables and lines for both PCA and CVA, we can assume that CVA had a higher capacity to account for higher variance in less variables.

Udeh and Ogbu (2011) found for CP1 high correlations for breast width, wing length, and thigh length, whereas for CP2 it had high correlation for drumstick length in the Arbor Acre line. In the Marshal line, there was a positive and high correlation for shank length, wing length and negative correlation on drumstick length to CP1. In CP2 there was a positive and high correlation for breast width, and a negative correlation for thigh length. CP3 had a high correlation with body length. And in the Ross line, for the first CP, there was a high positive correlation for breast width, body width, and negative for body

length. The CP2 responded mostly by wing length and tight length, while the CP3 had positive correlation only by drumstick length. The pattern that we can verify both in the 3 lines that Udeh and Ogbu used, as in the present study, is that the chest yield has positive correlation and high influence in the first CP, some lines present in CP1, others in CP2. However, the differences between the other variables that Udeh and Ogbu indicated to have a high correlation, and those of this research, may be due to the absence of compositional characteristics, and not using performance characteristics but linear measurements.

In Ajayi et al. (2012), CP1 had greater weights of Wing Length (WL), Wing Span (WS), Tail Length (TL), Body Length (BL) and Breast Girth (BG) in the lines Normal Feathered and Frizzle Feathered. In the naked neck line, the characteristics of BL and BG were more relevant in the first component, while in the line "Anak Titan" were the characteristics WL, WS, BL and BG. In the lines Normal Feathered and Frizzle Feathered, the characteristics SL and KL were those of greater interest in CP2 and CP3 respectively. In the naked neck line were the characteristics of WL, WS and SL in CP2, and KL in CP3. The characteristics SL and TL were more associated to CP2, whereas only KL had influence in CP3 in the line "Anak Titan".

In the first CV, Rosário et al. (2008) found higher weights for live weight and carcass weight and in both CV, found a high influence of live weight, suggesting that this characteristic would be fundamental in genetic divergence among broilers. The results of Rosário et al. (2008) for the characteristics of greater influence may have diverged due to using weights instead of yields, as well as only characteristics related to weights and not quality being used. Almeida et al. (2013) obtained as characteristics of greater influence in the first CV the carcass weight, thigh weight and breast width. Both carcass and breast were also characteristics of highly influential in the variance.

The results from Table 6 show that the TRC line is the line with the highest average for PC1, indicating that it is the line with the highest values for cooking loss. In contrast, the GNG line is the one with the lowest scores for this same variable. For PC2, the line with the highest value was CG, showing that it was the line with the best breast yield, but as a consequence, lower  $b^*$  and  $L^*$ . On the other hand, the CBC line has the lowest PC2, indicating lower breast yield, and higher carcass color. When analyzing table 7, we conclude that CBC was also the line with the lowest values of CV1, indicating low carcass and breast yields and higher. However, both lines PS and CG were not significant different, being the lines with the highest mean of CV1, indicating high breast and carcass yield and low  $L^*$  and  $b^*$ .

Using both graphs in figures 1 and 2, we can assume that the best lines when breeding for more carcass and breast yields would be PS, CG, CPK and PP. However, these 4 lines have less color in their carcass. The difference between them would be in the variables cooking loss and back yield. CG and PP would be the lines with lowest cooking loss between the four, CPK and CG would be the lines with the highest back yield between those same 4. Analyzing all the 4 lines, CG would be the best for all the 4 variables with most significant influence in the variance. PP would be the line with good breast and carcass yield and good cooking loss, but with negative back yield and low  $L^*$  and  $b^*$ . PS would be the line with good carcass and breast yield, but negative cooking loss and back yield, and low scores on color. And CPK line has good carcass, breast and back yield, but negative cooking loss with low scores in the color indexes.

However, it is worth noting that genotypes GNG, TRC and CBC may have their market since even with lower breast yield, the carcass of these birds has higher coloration. The differences between them would be in the cooking loss and back yield. Both GNG and CBC are lines with lower cooking loss scores, however GNG has high back yield, while CBC has a negative back yield. The TRC line has more breast and carcass yield than GNG and CBC, but also has negative scores in cooking loss and back yield.

## 5. Conclusion

The results of the multivariate analysis allowed the identification of the variables that interfere in the genotype difference, and it was an efficient methodology for the simultaneous interpretation of several characteristics in the genotype comparison analysis. Using both analysis we can conclude that the variables carcass yield, breast yield,  $L^*$  and  $b^*$  are the ones with most genetic divergence between lines. However, Cooking Loss and back yield are also characteristics that can cause divergence between genotypes, but with lower relevance than the other 4.

The seven genotypes evaluated can be grouped into three groups: 1- (PS, CG, CPK, and PP), 2- (GNG and CBC) and 3- (TRC). According to each of the groups it is possible to verify which are the most indicated genotypes according to the traits evaluated. This study showed that both PCA and CVA facilitate the interpretation of data with several traits of importance, demonstrating witch traits are the most responsible for genetic divergence.

These results emphasize the importance of using multivariate analysis to assess genetic divergence quality and carcass traits in order to identify which are the most relevant on those studied and to make simpler for readers to analyse what are the differences and similarities between genotypes evaluated. Both CVA and PCA have similarities and grant good results on data interpretation. Other methodologies of multivariate analysis that assume dependencies between traits, like Structural Equation Modeling or multivariate regression analysis, could be used in comparisons with the differences of PCA and CVA.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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