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Multivariate Analysis of the Relations Between Some Blood Biochemistry Parameters, Morphological Characters and Tonic Immobility in Broiler

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Multivariate multiple regression analysis (MMLR) and multidimensional scaling technique (MDS) were used to investigate relations between blood biochemistry parameters (white blood cells) and differences between two sides of some morphological characteristics (shank length, shank width, face width, face length) and tonic immobility. Sixty male birds were used in this study. According to the results of MMLR, the overall effect of basophil (P = 0.05), monocyte (P = 0.004) and eosinophil (P = 0.011) on dependent variables were statistically significant while the overall effect of lymphocyte (P = 0.630) and heterophil (P = 0.2937) were not statistically significant. Univariate F tests indicated that the shank width (P = 0.033) and face width (P = 0.047) were significantly related to the set of predictors, while shank length (P = 0.537), face length (P = 0.2344) and tonic immobility (P = 0.703) are not significantly related to the set of predictors. The MDS results showed that the differences between two sides of shank width were also related to Monocyte, Eosinophil and basophil while face length, face width and tonic immobility were related to lymphocyte and heterophil.

Key Words: Multivariate multiple regression analysis, Multidimensional scaling, Bilateral symmetry, White blood cell.

INTRODUCTION

It frequently made good use of the amount of deviation which observed on some morphological properties on determining the fear and stress upon animals, the tonic immobility period and the number of white blood cell[1-8]. This situation states the importance that the white blood cells and the morphologic properties mentioned before and the immobility period should be investigated in detail.

In practice, multiple linear regression analysis is commonly used to investigate the relations between the variables. In multiple linear regressions (MLR), however, the correlations among dependent variables are not considered[9,10]. Therefore, there is only one dependent variable which is related to several independent variables in multiple linear regression models. However, since the various dependent variables are correlated in general, a multivariate multiple techniques such as canonical correlation

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analysis (CCA), multivariate multiple linear regression analysis (MMLR) and multidimensional scaling (MDS) is necessary to be carried out, if one wish to get information on the correlation between the different dependent variables conditioned on the independent variables[11-15].

However, a limited number of traits and used univariate methods is available in the literature to analyze the data. This sort of approach does not account for correlations among the traits, which leads to lost information and the pre-determined Type I error rate (α) cannot be maintained[16]. Therefore, the experiments done to investigate the relations between many dependent and independent variables will be more accurate when all traits are evaluated simultaneously with multivariate analyses technique. As there are correlations among them, all traits considered to be essential. At the same time, the presentation of the analysis results on the multidimensional space will help both researchers and readers in terms of understanding and commenting the results easily[17,18].

This study was carried out with a view to investigating the relations between some of blood biochemistry parameters, morphological characters and tonic immobility value in broiler *via* two different multivariate techniques namely multivariate multiple regression and multidimensional scaling.

EXPERIMENTAL

A total of 60 broiler male chicks were used for 6 week trials. The chickens were raised under 17 birds/m^2 stocking density. Nipple drinker and round feeder were used to satisfy of water and feed requirement of birds. Water and feed was provided *ad libitum* with 23 h light and 1 h dark throughout the trial. Birds were fed with starter diet between 0-3 weeks and with growth diet between 4-5 weeks and with finisher diet in the last week of the trial. The starter, growth and finisher diets of the animals included 24.09 % crude protein, 2818 kcal/ME and 25.32 % crude protein, 2892 kcal/ME, 22.38 % crude protein, 2912 kcal/ME respectively under intensive condition. The following morphological characteristics (dependent variables) were measured when the birds were six weeks old: left and right shank width (SHW, mm), shank length (SHL, mm), face width (FL, mm), face length (FW, mm), tonic immobility (TONIC, sn) and number of white blood cells (independent variables) such as lymphocyte, basophil, heterophil, monocyte and eosinophil.

Blood samples were drawn from under-wing vein (*V. cutane ulnaris*). In counting white blood cells, the blood drawn from birds by means of a syringe was spread on a slide. After it dried, it was fixed by methanol and stained by Wright's dye. When it dried following buffering and washing, it was treated with xylol and then it was covered by entellen and made ready to observe by a microscope. The lymphocyte, heterophil, eosinophil, basophil, monocyte cells per 100 leukocyte cells were counted under microscopic conditions. Statistical analyses were performed with SAS Statistical Package Program[19].

Statistical analysis, multivariate multiple linear regression analysis (MMLR): Multivariate multiple linear regression analysis (MMLR) was used to investigate the relations between two data sets (dependent and independent variables). In this study, the first data set formed by blood biochemistry parameters (independent or predictor variables) and the second data set formed by some morphological characteristics and tonic immobility (dependent or response variables). The hypothesis being tested by a multivariate regression is whether there is a joint linear effect of the set of independent variables on the set of dependent variables or not. Hence, the null hypothesis is that the slope of all coefficients is simultaneously zero[9,10].

In MMLR, q dependent variables ($Y_1, Y_2, ..., Y_q$) are to be predicted by linear relationships with r independent variables ($X_1, X_2, ..., X_r$). That is, we are interested in how the set of dependent variables are related to the independent variables. Therefore, the statistical model for the MMLR is:

$$Y_{nxq} = X_{nx(r+1)}B_{(r+1)xm} + E_{nxq}$$

where Y represents n observations of a q-dependent variable, X represents the design matrix of rank r+1 with its first column being the vector 1, B is a matrix of parameters to be estimated and E represents the matrix of residuals[20].

Multidimensional scaling (MDS): The purpose of MDS is to produce visualizations for the exploration of data. Such visualizations allow users to detect interesting latent structure[18,21-23]. Multidimensional scaling plots the objects on a map such that the objects which are very similar to each other are placed near each other on the map and objects which are very different from each other, are placed far away from each other on the map. That is, the points close to each other in the map indicate relationship between the pairs as well as similarity of behaviour with respect to the remaining variables or objects[13,24]. The points located in the graphs reproduce distances between each pair, controlled by the distance of each variable (object) with each one of the remaining variables (objects). According to distances, configuration distances are computed using the following linear regression equation:

$$\delta_{ij} = a + bd_{ij} + e \quad (1)$$

where a = constant, b = regression or slope coefficient and e = residual term.

The degree of correspondence between the distances of the points implied by MDS map and the matrix input by the user are measured (inversely) by a stress function. Kruskal[25] suggests that stress should be informally interpreted according to the following guidelines (Table-1).

TABLE-1
STRESS COEFFICIENTS FOR GOODNESS OF FIT

Stress	Goodness of fit
≤ 0.200	Poor
0.100 - < 0.200	Fair
0.050 - < 0.100	Good
0.025 - < 0.050	Excellent
0.000 - < 0.025	Perfect

RESULTS AND DISCUSSION

MANOVA tests for the hypotheses of no overall effect of predictors variables (X1, X2, X3, X4, X5) on dependent variables (Y1, Y2, Y3, Y4, Y5) indicated that the overall effect of X2 (Wilk's $\Lambda = 0.326$; P = 0.05), X4 (Wilk's $\Lambda = 0.214$; P = 0.004) and X5 (Wilk's $\Lambda = 0.2691$; P = 0.011) were statistically significant. On the other hand, the overall effect of X1 (Wilk's $\Lambda = 0.7383$; P = 0.6303) and X3 (Wilk's $\lambda = 0.5832$; P = 0.2937) were not statistically significant (Table-2). Therefore, parameters for dependent variables (Y1, Y2, Y3, Y4 and Y5) are not the same for the variable X2, X4 and X5, while that parameters are the same for X1 and X3 variables. The multivariate test determines whether there is a significant relationship between the two sets of variables, namely blood biochemistry parameters (dependent variables), morphological characters and tonic immobility (independent variables). The results of multivariate test indicated that there is a significant regression of the set of two dependent variables on the 3 predictors.

TABLE-2
MANOVA TEST FOR THE HYPOTHESES OF NO OVERALL Xi's EFFECT

Predictors	X1	X2	X3	X4	X5
Wilk's Λ	0.7383	0.3260	0.5832	0.2140	0.2691
P-value	0.6303	0.0500	0.2937	0.0040	0.0115

Univariate F tests are used to test the significance of the regression of each dependent variable separately. They indicated that shank width (Y2; P = 0.033) and face width (Y3; P = 0.047) are significantly related to the set of predictors, while shank length (Y1; P = 0.537), face length (Y4; P = 0.234) and tonic immobility (Y5; P = 0.703) are not significantly related to the set of predictors (Table-3). In other words, for the dependent variable shank length, face length and tonic immobility none of the predictors are statistically significant but for the shank width and face width, at least one predictor is statistically significant[26]. For the shank width, the effect of basophil, monocyte and eosinophil statistically significant ($P < 0.05$). For the face width, the effect of heterophil and monocyte are statistically significant ($P < 0.05$).

TABLE-3
REGRESSION EQUATIONS, R^2 (%) AND MSE VALUES

Variables	Regression equations	R^2	MSE	P
Shank length	Y1=-88.214+6.600X1+0.617X2-1.120X3+0.635X4-0.1155X5	23.3	3.34	0.537
Shank width	Y2=19.02-0.044X1-3.395X2+0.417X3+1.529X4-2.921X5	64.5	2.46	0.033
Face width	Y3=-42.25+0.930X1-0.045X2+1.676X3+1.151X4+0.142X5	56.2	1.48	0.047
Face length	Y4=0.663+0.371X1+0.364X2-0.712X3-0.266X4-0.316X5	35.8	3.65	0.234
Tonic immob.	Y5=-33.856+9.103X1+0.687X2-8.632X3-3.601X4-3.641X5	17.6	12.6	0.703

In this study, MDS technique is also used to investigate the relationships among the blood biochemistry parameters and left-right side differences of four morphological characteristics such as shank length, shank width, face width and face length and tonic immobility of birds. MDS is more appropriate technique especially when assumptions of parametric tests such as multiple regression, multivariate regression and canonical correlation analysis are satisfied.

Goodness of fit of MDS is evaluated by stress coefficient and R^2. In this study, R^2 and stress coefficient were found as 87.4 % and 0.094, respectively. Therefore, it could be said that MDS techniques were suitable to investigate the relationships between two data sets. In other words, MDS map has almost reproduced the input data of this study perfectly. In the MDS technique, the traits are placed on a map as objects (Fig. 1). When MDS map is examined it was seen that left-right differences for shank width (SHW) was related to monosit (MONO), eozonofil (EOZ) and basophil (BASO) while face width (FW) and face length (FL) and tonic immobility (TONIC) were related to LENF and HETE (Fig. 1). Hence, differences in left and right shank width of birds were mostly affected from monosit, eozonofil and basophil while the differences in left and right face length and face width and tonic immobility of the birds were related to Lymphocyte and heterophil value. On the other hand, shank length (SHL) variable established different pattern.

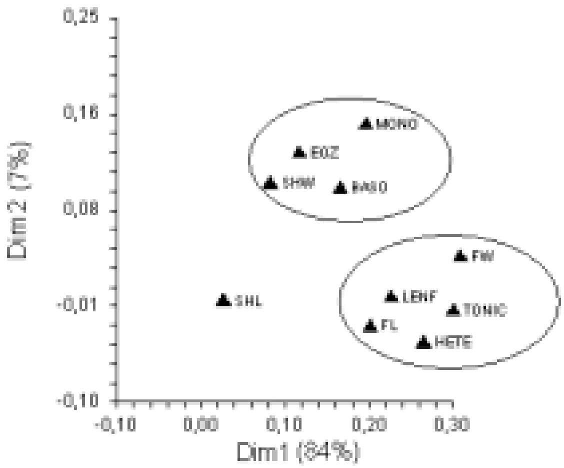

Fig. 1. MDS map for relations between the variables

Investigation of relations among variables is quite important for all area as well in animal breeding[14]. In order to state the relationships between the variables confidently, the relationships must be investigated with the aim of appropriate statistical techniques. In this study, MMRL and MDS techniques which are used to investigate the relationships between dependent and independent data set, gives such results which complements each other. So applying those two techniques together provides to evaluate the relationships between two variable data sets.

Conclusion

Multivariate multiple linear regression and multidimensional techniques can be a useful way of demonstrating the relationships between two variable sets in animal based studies as well as in other areas of research. By the aim of this motivation, it can be concluded that the variations in the amount of bosophil, monocyte and eosinophil can form shank-width deviations and, the variations in the amount of heterophil and monocyte can form face-width deviations. Consequently, as a result, the increase in quantitative amount of bosophil, monocyte, eosinophil and heterophil might cause unstable growth of shank-width and face-width with a high probability.

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