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Examination of Relationships Between Some Biochemical and Oxidative Stress Traits by Canonical Correlation Analysis in Broiler Chickens

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A R T I C L E I N F O

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A B S T R A C T

Canonical correlation analysis is a multivariate method to examine the relationships between two (X and Y) sets of variables when all measurements are obtained from same broilers. Canonical correlation analysis aims to obtain new variables called as canonical variates formed by linear combinations of the original variables for each set and by maximizing the relationships between two set. The purpose of this study is to examine the relationships between 8 biochemical traits (Aspartate Aminotransferase (AST), Albumin, Triglyceride, Total Cholesterol, Low Density Lipoprotein (LDL) cholesterol, Glucose, Total Protein and Alanine Aminotransferase (ALT)) and 4 oxidative stress traits (total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), lipid peroxide (LPO)) in broiler chickens. As a result, the correlation between the first canonical variable pair was found 0.594.

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Etlik Piliçlerde Kanonik Korelasyon Analiziyle Bazı Biyokimya ve Oksidatif Stres Parametreleri Arasindaki İlişkinin Tahmini

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Ö Z E T

Kanonik korelasyon analizi, tüm ölçümlerin aynı etlik piliçlerden elde edildiğinde iki değişken kümesi arasındaki (X ve Y) ilişkiyi inceleyen çok değişkenli bir istatistik yöntemdir. Kanonik korelasyon analizi, her küme için orijinal değişkenlerin doğrusal kombinasyonlarıyla oluşturulan kanonik değişkenler olarak adlandırılan yeni değişkenleri elde etmeyi ve iki küme arasındaki ilişkileri en üst düzeye getirmeyi amaçlamaktadır. Bu çalışmanın amacı, etlik piliçlerde 8 biyokimyasal özellik (aspartat aminotransferaz (AST), Albumin, Trigliserid, Toplam Kolesterol, Düşük Yoğunluklu Lipoprotein (LDL) Kolesterol, Glukoz, Toplam Protein ve alanin aminotransferaz (ALT)) ile 4 oksidatif stres özellikleri (toplam antioksidan statüsü, toplam oksidasyon statüsü, oksidatif stress inedeksi, lipid peroksit) arasındaki ilişkiyi incelemektir. Sonuç olarak, ilk kanonik değişken çift arasındaki korelasyon 0.594 olarak bulunmuştur.

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Introduction

There are considerable relationships between biochemical and oxidative stress traits. In general, several univariate (relationships) measurements such as Pearson correlation and regression coefficients are used to determine of these relationships. However, for determining of the relationships by this approach, only two variables are considered and the effects of other variables on these relationships are ignored. Thus, whole relationships structure may be impaired. Instead of univariate methods, using of multivariate methods can provide more information. Canonical correlation analysis is one of the common multivariate methods and employed to examine the relationships between two variable sets contained at least two or more variables.

The objective of this study is to examine relationships between some biochemical and oxidative stress traits in broiler chickens.

Materials and Methods

Material

Material of this research consists of 120 broilers. 12 traits were measured from these broiler chickens. 8 of these traits were grouped into X variable and the rest of (4) into Y variable. These traits are AST, Albumin, Triglyceride, Total Cholesterol, Low Density Lipoprotein (LDL) Cholesterol, Glucose, Total Protein (TP), ALT, Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Oxidative Stress Index (OSI), Lipid Peroxide (LPO)

Methods

Let these two sets be

$$X\left(\acute{X} = [X_1\ X_2\ \cdots X_P]\right)$$

and

$$Y\left(\acute{Y} = \left[Y_1\ Y_2\ \cdots Y_q\right]\right)$$

of dimension m x p and m x q and the data in $X_{m\ x\ p}$ and $Y_{m\ x\ q}$ sometimes are called the independent and dependent variables, respectively. The maximum number of correlations found between two sets is then equal to the minimum of the column dimensions p and q. We search for maximal correlations between the two subsets of variables by considering linear combinations;

U=$\acute{a}X$ and V=$\acute{b}Y$ of the X's and Y's, respectively.

We then have that

$\sigma_U^2 = \acute{a}\sum_{XX} a$, $\sigma_V^2 = \acute{b}\sum_{yy} b$ and $\sigma_{UV}^2 = \acute{a}\sum_{XY} b$

Hence,

$$Corr(U,V) = \frac{\acute{a}\sum_{XY} b}{\sqrt{\acute{a}\sum_{XX} a}\sqrt{\acute{b}\sum_{yy} b}} \quad (1)$$

The problem is now to estimate a and b that maximize equation (1) given the assumpions below:

$\sigma_U^2 = \acute{a}\sum_{XX} a$=1 and $E(U) = E\left(\acute{a}X\right) = \acute{a}E(X) = 0$ (2)

$\sigma_V^2 = \acute{b}\sum_{yy} b$=1 and $E(V) = E\left(\acute{b}Y\right) = \acute{b}E(Y) = 0$ (3)

Let the maximization problem of eq. (1) write in Lagrangian form by using two constrains (2) and (3):

$$L(\lambda_X, \lambda_Y, \mathbf{a}, \mathbf{b}) = \mathbf{a}'\mathbf{\Sigma}_{XY}\mathbf{b} - 0.5\lambda_X(\mathbf{a}'\mathbf{\Sigma}_{XX}\mathbf{a} - 1) - 0.5\lambda_Y(\mathbf{b}'\mathbf{\Sigma}_{YY}\mathbf{b} - 1) \quad (4)$$

In order to maximize the eq. (4), after taking derivatives $L(\lambda_X, \lambda_Y, a, b)$ with respect to a and b, the resulting equations are presented in the matrix form:

$$\begin{bmatrix} -\lambda\sum_{XX} & \sum_{XY} \\ \sum_{YX} & -\lambda\sum_{YY} \end{bmatrix}\begin{bmatrix} a \\ b \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \quad (5)$$

with the constraint $\lambda_X = \lambda_Y = \lambda$ given eq. (2) and (3). Hence, canonical correlations are estimated from the highest one to lowest one ($\lambda_1 \geq \lambda_2 \geq \cdots \lambda_P$) which are p roots of the determinant of coefficent matrix in eq. (5) (Tabachnick and Fidell, 2001; Johnson and Wichern, 2002; Keskin and Ozsoy, 2004).

Testing of significant canonical correlations are required and Bartlett test is a very common test (Thompson, 1985). In this test, X^2 test statistic is computed as follows:

$$X^2 = [n - 0.5(V_1 + V_2 + 1)] \times \log(\Lambda)$$

where n: number of observations, V_1 and V_2: number of variables in the sets of X and Y and

$$\Lambda = (1 - R_{k1}^2)(1 - R_{k2}^2)\cdots\left(1 - R_{kp}^2\right),$$

then is compared with $X_{p\times q}^2$ table value. In this procedure, if we reject that H_0: all canonical correlations = 0, the largest correlation coefficent is extracted and the test is repeated until we fail to reject H_0, which means that all significant correlations are determined. Statistica for Windows (release 7.0) statistical packet program was used for all of the calculations (StatSoft, 2004).

Results and Discussion

Descriptive statistics for the studied traits were presented in Table 1 and Pearson correlation (r) coe In Table 1, descriptive statistics of the X and Y variable sets are given. The highest variation in X variable set has LDL cholesterol (35,248%); highest variation in Y variable set has OSI and TOS (57,991% and 57,194%). Pearson corelation coefficients (r) were given in Table 2. Table 2 shows that the highest correlation was found for total cholesterol and LDL cholesterol (r = 0.831) in X variable set; OSI and TOS in Y variable set (r = 0.920); in the X and Y varieties, TAS and triglyceride (r = 0.290) are observed.

Table 1 Descriptive statistics for studied variables

Biochemical parameters	Mean	Std. Dev.	Min.	Max.
AST	328.051	88.687	208	769
Albumin	1.549	0.332	0.70	2.50
Triglyceride	513.043	78.357	22	2146
Total Cholesterol	207.778	63.009	109	448
LDL Cholesterol	126.889	44.726	56	303
Glucose	328.345	35.576	251	448
Total Protein	3.039	0.972	1.50	6
ALT	6.593	2.072	2.00	12
TAS	1.947	0.430	1.14	2.75
TOS	4.177	2.389	1.07	14.95
OSI	0.219	0.127	0.06	0.67
LPO	0.127	0.041	0.07	0.24

Table 2 Pearson correlation coefficient for traits in two sets

	AST	ALB	TRG	TCH	LDL	GL	TP	ALT	TAS	TOS	OSI	LPO
AST	1											
ALB	0.208*	1										
TRG	0.202*	0.477**	1									
TCH	0.261**	0.220*	0.373**	1								
LDL	0.170	0.135	0.515**	0.831**	1							
GL	0.299**	0.102	0.161	0.401**	0.290**	1						
TP	0.064	0.444**	.464**	.431**	0.453**	0.211*	1					
ALT	-0.224*	-0.063	-0.122	-0.138	-0.026	-0.113	0.033	1				
TAS	-0.010	0.107	0.290**	0.057	0.177	-0.049	-0.030	0.062	1			
TOS	0.123	-0.069	.033	-0.074	-0.043	-0.095	-0.016	-0.007	0.166	1		
OSI	0.116	-0.088	-0.022	-0.086	-0.073	-0.060	0.047	-0.035	-0.186*	0.920**	1	
LPO	0.139	-0.041	-0.005	-0.121	-0.077	-0.154	0.027	-0.038	-0.071	0.820**	0.865**	1

ALB: Albumin, TRG: Triglyceride, TCH: Total Cholesterol, LDL: LDL Cholesterol, GL: Glucose, TP: Total Protein, * P<0.05; ** P<0.01; LDL: Low Density Lipoprotein, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, LPO: Lipid Peroxide

Table 3 Canonical correlation coefficients

Canonical		P value	Wilk's Lambda
Variables	Correlations		
U_1V_1	0.594	0.004	0.437
U_2V_2	0.428	0.280	0.676
U_3V_3	0.319	0.498	0.875
U_4V_4	0,159	0.738	0.974

Table 4 Standardized canonical coefficients and canonical loadings for the first canonical variate pairs

Biochemical parameters	Standardized Canonical Coefficients	Variable - Variate Correlations	
		U_1	V_1
AST	-0.371	-0.196	-0.079
Albumin	0.318	0.382	0.154
Triglyceride	0.236	0.591	0.238
Total Cholesterol	-0.990	0.263	0.106
LDL Cholesterol	1.199	0.621	0.250
Glucose	0.376	0.292	0.117
Total Protein	0.142	0.527	0.212
ALT	-0.097	0.035	0.014
TAS	2.024	0.219	0.545
TOS	-4.244	-0.078	-0.193
OSI	4.447	-0.093	-0.231
LPO	-0.464	-0.093	-0.231

LDL: Low Density Lipoprotein, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, LPO: Lipid Peroxide

As seen in Table 2, most of the correlation coefficients between the variables were found statistically significant at 1% or 5% level. The highest correlation coefficient was observed between OSI and TOS.

In this study, X and Y variable sets had $p = 8$ and $q = 4$ variables, respectively. Thus, four canonical variable or variate pairs (U_i V_i) can be potentially extracted and canonical correlations between them were computed by using eq. (1). These canonical correlations were presented in Table 3.

As seen in Table 3, only the first canonical correlation between U and V canonical variate pairs was found statistically significant ($P<0.05$). Thus, only the first canonical variate pairs was considered further analysis. According to first canonical variate pairs (U_1V_1), the canonical correlation is 59.4% ($r_{U1V1} = 0.594$)]. This result indicated that investigation of the relationships between biochemical and oxidative stress traits in broilers by using first canonical variates (U_1 and V_1) will be equivalent to original variables. Thus 35.28 ($=0.594^2$) percent of the variation in 12 original variables will be explained by only U_1 and V_1 canonical variates.

Table 4 shows the standardized canonical coefficients. Standardized canonical coefficients can be interpreted as multiple regression coefficients in the multiple regression analysis. In canonical correlation analysis, standardized canonical coefficients show the change in canonical variable in terms of their standard deviation when original variable changes one standard deviation. In other words, these coefficients indicate the effect of original variables on the canonical variates. These coefficients and variable - variate correlations or canonical loadings were presented in Table 4.

From the Table 4, equations can be written in terms of standardized canonical coefficients for U_1 and V_1 canonical variate pairs as following:

$$U_1 = -0.371\ AST + 0.318\ ALB + 0.236\ TRG - 0.990\ TCH + 1.199\ LDL + 0.376\ GL + 0.142\ TP - 0.097\ ALT \quad (6)$$

(ALB: Albumin, TRG: Triglyceride, TCH: Total Cholesterol, LDL: LDL Cholesterol, GL: Glucose, TP: Total Protein)

$$V_1 = 2.024\ TAS - 4.244\ TOS + 4.447\ OSI - 0.464\ LPO \quad (7)$$

For U_1 variate LDL (1.199) had the highest coefficient in X set. Similarly, the coefficient of OSI (4.447) was the highest one in Y set. On the contrary, standardized coefficient of GPT (-0.097) in X set was negative and had very low effect on U_1 canonical variate. However, standardized canonical coefficients can be unstable for small sample size and for the presence of multicollinearity in the data. For this case, Sharma (1996) suggests the use of correlation between canonical and original variables which is called loading or structural correlation. Thus, loadings for first canonical variables were computed and given also in Table 4.

Loadings of all original variables, except AST, in X set were found positively correlated with U_1 and V_1. However, all loadings in Y set, except TAS were negatively correlated with U_1 and V_1

Although, canonical coefficient of OSI was positive and high, canonical load of this variable was found negative and low.

When considered the loadings of the original variables in X set, LDL cholesterol had the highest value with 0.621 and this followed by Triglyceride with 0.591, Total Protein with 0.527 while AST had negative and lowest value (-0.196). Similarly, in Y set, TAS was highly and positively correlated with V_1 canonical variate while the smallest value (-0.193) belonged to TOS.

In order to obtain high value for U_1 canonical variate, AST should be lower value. However other variables need to be high values. Similarly, in order to obtain high value for V_1 canonical variate, all of the oxidative stress traits, except TAS should have low values.

Canonical correlation analysis was carried out for determination the relationships between biochemical and oxidative stress traits. According to results of this analysis, linear relationship between the two-variable set was determined as 59.4% (Figure 1). Thus, it can be highly expected that when biochemical traits have high values, oxidative stress traits also will be high.

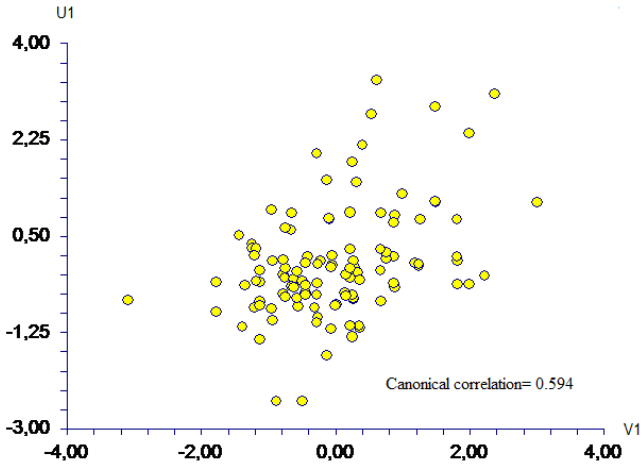

Figure 1 Scater plot of canonical variates U1 and V1

U1 will be increased when V1 is increased because the canonical coefficient between U1 and V1 canonical variables is positive. According to this, the increase of serum albumin, triglyceride, LDL cholesterol, glucose and total protein will cause to the increase of V1 and as a result of this, TAS and OSI will be increased. Values with negative coefficient in V1 also will be decreased while values with negative coefficient in U1 are reduced. So, the reduction of serum AST and total cholesterol level caused to the increase in TAS and OSI, and the reduction of TOS and LPO.

The increase of TAS enhanced serum albumin and total protein levels of quails. This increase might be derived from the reduction of synthesis and secretion of corticoid hormones in quails due to increasing TAS. The reduction of corticoids' levels might have decreased protein catabolism. As a result, serum albumin and total protein levels were increased (Seyrek et al., 2004).

Despite the increasing TAS of quail, the enhancement of serum glucose level might be derived from an increase in free radicals and the release of stress hormones such as

ACTH and corticol that prevent insulin release (Ajakaiye et al., 2010). The increase of TAS did not may have been enough for prevention the release of stress hormones.

The higher levels of stress hormones in circulating system might have stimulated lipolysis and increased triglyceride levels in serum although serum TAS was increased (Hajati et al., 2016).

Increasing TAS and decreasing TOS reduced liver AST and ALT enzymes' levels. The increase of TAS protects liver from the harmful effects of oxidative stress.

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