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Path coefficient analysis of haemato-biochemical traits to explore the heat stress in native Khadia chicken population of northern Odisha, India

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ABSTRACT

The aim of the present study was to evaluate the haemato-biochemical parameters of native Khadia chicken reared under backyard poultry production system. Data were collected from 280 Khadia chicken population. The haemato-biochemical parameters were altered significantly due to heat stress. The stress related traits, viz. heart rate (447.68±4.50 beat/min) and cloacal temperature (46.32±0.12) were recorded. The correlation between heart rate and AST was 0.76 and the correlation with other haemato-biochemical parameters was also significant under stressful conditions. Similarly, cloacal temperature also significantly correlated with haemato-biochemical parameters under thermal stress. The path coefficient analysis was executed to identify the direct effect of AST on heart rate and cloacal temperature which were 0.57 and 0.46, respectively. The multiple regression analysis showed AST is the central factor contributing to variation in stress related traits which can be used as a selection tool for selection at early stage of birds for thermal tolerance.

Keywords: Direct effect, Heat stress, Khadia chicken, Path analysis, Regression

Since time immemorial, Khadia tribal communities inhabited the Northern districts namely, Mayurbhanj and Keonjhar of Indian State of Odisha. They have been raising a particular native chicken population (Khadia) irrespective of culture and religion with distinct phenotypic characters (Dessie *et al*. 2012, Singh *et al*. 2016) for generating family income and sustaining their livelihood (Mohapatra and Panda 1981, Padhi 2016, Parveen *et al.* 2017, Nayak *et al*. 2020). The native Khadia birds are usually raised in backyard poultry production system with low or limited inputs, and play a vital role in providing livelihood and food security to the rural families as being practised in many developing and underdeveloped countries (Aini 1990, Barua and Yoshimura 1997, Khan 2008, Reta 2009, Magothe *et al.* 2012, Maddheshiya *et al.* 2020). Heat stress (HS) in chickens is the major global concern in the poultry production, since it causes high mortality and reduces growth, especially during the hot season, and mitigation strategies have been architected to overcome the future challenges facing the global poultry industry (Nawab *et al.* 2018). Several studies have investigated the detrimental effects of heat stress on poultry production and it has been revealed that heat stress negatively affects the welfare and productivity of broilers and laying hens as well as livestock production system (Aengwanich 2007, Nienaber and Hahn 2007, Renaudeau *et al.* 2012, Lara and Rostagno 2013, Saeed *et al.* 2019).

Heat stress (HS) is a commonly encountered challenge of the poultry production industry, especially in regions with tropical and subtropical climates (Faragand 2018, Mosleh *et al.* 2018). Tropical climate affects poultry production due to long duration of summer season. As chickens have faster body metabolism, they are mostly affected by HS owing to higher body temperature and without sweat gland (Al-Aqil and Zulkifli 2009). The combination of high humidity and temperature become critical as birds evaporate heat with much difficulty (Gaviol *et al.* 2008). HS increases mortality rate, reduces production, decreases feed intake and lowers body weight (Cooper and Washburn 1998, Akaboot *et al.* 2010, Boonkum *et al.* 2014). Although rearing of indigenous chickens is profitable for the tribal population worldwide but, heat stress during the summer season drastically reduces their profitability thereby severely affecting their livelihood (Kamboh *et al.* 2013, Ranjan *et al.* 2019). The heart rate (HR) and cloacal temperature (CT) are the primary indicators of HS in birds (Nascimento *et al.* 2012, Nascimento and Martins 2017). The haemato-biochemical parameters also change during HS (Khan *et al.* 2002, Adekunle *et al.* 2017).

Path coefficient estimates direct and indirect effect of independent traits on dependent traits. It also estimates the residual effects of related traits on dependent traits. The computed path coefficient indicates the amount of change

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expected in the dependent variable as a result of a unit change in the independent variable (Smith *et al.* 1997). The characters that are analyzed in path coefficient can be utilized to develop a multiple regression equation model to predict the future behaviour of chicken during HS. There is limited documentary evidence of relationship between stress related traits with haemato-biochemical parameters through path analysis and regression equations. There is no literature available on path coefficient analysis of haemato-biochemical traits in HS condition in native chickens. Thus, to improve the productivity and efficiency of the native chicken population within their local environments, the present path coefficient analysis was undertaken to determine the direct and indirect effects of haemato-biochemical parameters on stress related traits, and futuristic regression model in native Khadia chicken of Northern Odisha with a view to yielding more appropriate selection criteria.

MATERIALS AND METHODS

Study area: The present research work was conducted in two districts namely, Mayurbhanj and Keonjhar in the Indian State of Odisha. The study area villages are situated at 12°15′N to 13°00′N and 39°10′E to 39°50′E with altitude ranging from 930 to 3925 masl. The mean temperature varies from 9–28°C and the mean annual rainfall ranges from 400–912 mm (SZT). Adult native Khadia chickens (280), equally from both the sex were selected randomly from these two districts. The existence of unique variety of Khadia populations, good transportation access, better co-operation from the poultry owners were some of the important factors to carry out the present study in these areas.

Recording of stress related traits: HR and CT were measured as stress related traits both in thermal comfort (TC) and HS conditions. A stethoscope and a stop watch were used to measure the heart rate by counting the number of beats per minute. A digital thermometer was used to measure the CT by inserting it 3 cm into the rectum.

Sample collection: Blood samples (3 ml) were collected from adult birds at farmers' doorsteps by puncturing the wing vein with sterile syringes and needles (24-gauge) with anticoagulant for haematological analysis and without any anticoagulant for biochemical analysis following the standard procedure.

*Estimation of serum AST and ALT :*The serum samples were used to estimate the Aspartate Aminotransferase (AST) through Mod. IFCC method using commercial kit (CORAL^R) (IFCC 1986b). Alanine Aminotransferase (ALT) was estimated by Mod. IFCC method using commercial kit (CORAL^R) (IFCC 1986a). The measurement of AST and ALT was performed using UV-Vis spectrophotometer (M/s Chemiline Technologies Ltd., India).

Estimation of Hb and PCV: Hemoglobin (Hb) content was determined using Sahli's hemoglobinometer (Jain 1993) and packed cell volume (PCV) was estimated by Wintrobe's Haematocrit method (Jain 1993).

Statistical analyses: The paired sample t-test was used to find the significant mean difference between two sets of observations of stress related traits with haemato-biochemical parameters. The association between stress related traits with haemato-biochemical parameters was estimated by Pearson's correlation method (Snedecor and Cochran 1989). The direct and indirect effect as well as residual effect were also determined employing path analysis (Singh and Choudhary 1985).

Path analysis: Direct and indirect effects of the AST, ALT, Hb and PCV on HR and CT during HS and TC condition were determined (Singh and Choudhary 1985). AST, ALT, Hb and PCV are independent variables and heart rate and CT dependent variables. The direct and indirect effects were calculated, each for heart rate and CT separately in TC as well as HS conditions as per Singh and Chaudhary (1985).

The residual effects during HS and TC condition were also estimated in the present experiment (Singh and Choudhary 1985). A multiple regression equation was developed using independent and dependent variables to formulate future stress status of the native chicken (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Haemato-biochemical parameters as well as stress related traits were estimated both during TC and HS conditions in Khadia chicken population (Table 1).

Table 1. Effect of thermal conditions on AST, ALT, Hb and PCV

Parameter	TC condition	HS condition
AST (µ/l)	173.88±0.64^a	216.62±1.3^b
ALT (µ/l)	21.60±0.28^a	49.69±0.49^b
Hb (g %)	10.29±0.10^a	17.78±0.19^b
PCV (%)	36.67±0.20^a	56.13±0.42^b
HR (beats/min)	258.14±6.6^a	447.68±4.5^b
CT (°C)	40.48±0.16^a	46.32±0.12^b

Values are Mean±SE (n=280). Values having different superscript (a, b) within a row differ significantly (P<0.01).

All the haemato-biochemical parameters along with stress related traits were significantly higher during HS condition (P<0.01). Heat stressed birds showed an increased level of liver enzymes in serum which could be correlated with higher mortality rate. Since, the haemato-biochemical parameters play vital role for the faster and realistic evaluation of multiple organ dysfunctions, their alteration recorded in our study under thermal conditions could have a significant diagnostic aid of liver dysfunction, which corroborates with the finding of Huang *et al.* (2018). AST and ALT under TC and HS in Himachal differed from the present finding, which may be due to extremely low temperature and high altitude (Saklani *et al.* 2019). AST in the present finding is in consonance with Abd El-Hack *et al.* (2018) who observed significantly increased (P<0.01) serum AST but significant decrease (P<0.01) in ALT activity while using vitamin A supplementation under heat stress condition. Ladokun *et al.* (2008) reported that the Hb and PCV were 11.60 g/dl and 35.90%, respectively, in the normally feathered Nigerian native chicken. The Hb (10.29±0.10 g%) and PCV (36.67±0.20%) in the present finding are in agreement with findings of Dutta *et al.* (2013), Bora *et al.* (2017), Barik *et al.* (2019) and Duah *et al*. (2020).

The present Hb value is in contrast to the finding of Jaiswal *et al.* (2017) who reported a significantly decreased (P<0.05) value of Hb in CARIBRO-Vishal broiler chicken which may be attributed to breed difference and managemental practices. Characteristically, the determination of hemato-biochemical changes are obligatory to understand the various physio-biochemical conditions of the body and also for the determination of environmental stress, pathological, and nutritional factors responsible for altering these parameters (Pollock *et al.* 2001, Islam *et al.* 2004). It is necessary for the excess temperature to be dissipated from the body to surrounding environment through cellular conduction and vascular convection to maintain normal body temperature (Scanes 2015).

Correlation among AST and ALT with Hb and PCV in TC and TS conditions determined that AST and ALT values were negatively correlated with Hb and PCV values without any statistical significance during TC condition (Table 2). However, during HS condition, AST and ALT values were positively correlated with Hb and PCV (Table 2). In the HS condition, HR and CT were positively correlated (P<0.01) with AST, ALT, Hb and PCV with high statistical significance.

Path analysis: The Hb value was positively correlated with heart rate and the direct effect of Hb on HR was moderately positive (Table 3). The indirect effect of PCV through ALT was positive (Table 3). In TC condition, AST and PCV values were negatively correlated with heart rate and the direct effect of these on HR was negative (Table 4). The ALT value was negatively correlated with HR and direct effect of ALT on HR was positive (Table 4).
In TC condition, AST, ALT and PCV values were positively correlated with CT and the direct effects were positive. The Hb value was negatively correlated with CT and the direct effect of Hb on CT was moderately positive.

Direct and indirect effect of haemato-biochemical parameters on heart rate and CT under HS conditions demonstrates that in HS condition, AST and PCV values were positively correlated (P<0.01) with heart rate with direct effect being highly positive (Table 4). The ALT and Hb values were positively correlated with heart rate with direct effect being negative (Table 4). On the other hand, in HS condition, AST, Hb and PCV values were positively correlated with CT with direct effect being positive (Table 4). The ALT value was positively correlated with heart rate with direct effect being negative (Table 4). HS undesirably affects the productive and reproductive performance, economic traits and welfare of poultry (Oguntunji and Alabi 2010, Yousaf *et al.* 2019). During HS condition, the correlation between heart rate and AST was 0.763, whereas the direct effect of AST on heart rate was 0.574 indicating both are high in nature. AST, an enzyme found mostly in heart and liver, is secreted in elevated amount during stress.

Table 2. Correlation amongst hemato-biochemical parameters in TC and TS conditions

Climatic condition	Haemato-biochemical parameter	AST	ALT	Hb	PCV
TC	AST (μ/l)		0.182	–0.184	–0.057
	ALT (μ/l)	0.182		–0.162	–0.072
	Hb (g %)	–0.184	–0.162		–0.034
	PCV (%)	–0.057	–0.072	–0.034	
	HR (beats/min)	–0.106	–0.198	0.116	–0.101
	CT (°C)	0.211	0.257	–0.045	0.173
TS	AST (μ/l)		0.344*	0.463**	0.549**
	ALT (μ/l)	0.344*		0.378**	0.354*
	Hb (g %)	0.463**	0.378**		0.368**
	PCV (%)	0.549**	0.354*	0.368**	
	HR (beats/min)	0.763**	0.305*	0.334*	0.700**
	CT (°C)	0.655**	0.294*	0.447**	0.533**

Values are correlation between the parameters (n=280). *Correlation is significant at the 5% level of significance (2-tailed). **Correlation is significant at 1% level of significance (2-tailed).

Table 3. Direct and indirect effect of haemato-biochemical parameters on HR and CT under TC condition

Parameter	Correlation with HR	Direct effect	Indirect effect				Correlation with cloacal temperature	Direct effect	Indirect effect			
			AST	ALT	Hb	PCV			AST	ALT	Hb	PCV
AST (μ/l)	–0.106	–0.172		0.015	–0.072	0.014	0.211	0.169		0.047	–0.065	–0.012
ALT (μ/l)	–0.198	0.083	–0.031		–0.064	0.014	0.257	0.257	0.031		–0.057	–0.012
Hb (g %)	0.116	0.393	0.032	–0.013		0.018	–0.045	0.352	–0.031	–0.042		–0.015
PCV (%)	–0.101	–0.250	0.010	–0.006	–0.013		0.173		–0.010	–0.019	–0.012	

Residual (h) (effect of other variables on HR) during TC condition = 0.86

Residual (h) (effect of other variables on cloacal temperature) during thermal comfort condition = 0.88

Values are correlation as well as direct and indirect effects (n=280).

Table 4. Direct and indirect effect of haemato-biochemical parameters on CT and HR under HS condition

Parameter	Correlation with CT	Direct effect	Indirect effect				Correlation with HR	Direct effect	Indirect effect			
			AST	ALT	Hb	PCV			AST	ALT	Hb	PCV
AST (μ/l)	0.655	0.463		–0.001	0.070	0.123	0.763	0.574		–0.003	–0.038	0.230
ALT (μ/l)	0.294	–0.002	0.159		0.057	0.123	0.305	–0.010	0.197		–0.031	0.230
Hb (g%)	0.447	0.151	0.214	–0.001		0.079	0.334	–0.082	0.266	–0.004		0.148
PCV (%)	0.533	0.224	0.254	–0.001	0.056		0.700	0.419	0.315	–0.004	–0.030	
Residual (h) (effect of other variables on CT) during HS condition = 0.73							Residual (h) (effect of other variables on HR) during HS condition = 0.52					

Values are correlation as well as direct and indirect effects (n=280).

So, positive correlation was found and the direct effect was very high (0.574) during HS and AST may be taken as selectable trait to minimize HS. The correlation between HR and ALT during HS was 0.305 whereas its direct effect was –0.01 which is negligible (Table 4). The indirect effect of ALT on HR seems to be critical on HS. Hence, ALT may be included in a simultaneous selection model along with other direct selectable traits. Similar finding with elevated values of AST and ALT during HS was observed in earlier study (Khan *et al.* 2002) that supports the present finding.

The Hb gene expression is regulated by stress (Ilan *et al.* 2017). The correlation between heart rate and Hb during HS was 0.334 whereas its direct effect was –0.082 (Table 4). So the indirect effect of Hb on heart rate seems to be important and cause of the correlation. The indirect effect of Hb through AST, ALT and PCV were 0.266, –0.004 and 0.148, respectively (Table 4). So Hb again may be included in a simultaneous selection model along with other direct selectable traits. Upon induction of acute stress, cell produce proteins such as heat shock proteins and chaperons which lead to an increase in PCV (Iwama *et al.* 1999). The correlation between heart rate and PCV is 0.7 whereas the direct effect of PCV on heart rate is 0.419 (Table 4) indicating both are high in nature during HS condition. A direct selection through PCV to increase heart rate in order to minimize the HS may be effective. PCV can be taken as direct selectable trait.

Table 4 shows the residual effect of other variables on HR during HS which is equal to 0.52. Its complement (1-h) takes the value of 0.48. The AST, ALT, Hb and PCV explain only 48% of the variability in HR whereas factors other than mentioned above explain only 52% of variability in HR during HS (Singh and Choudhary 1985). Thus a simultaneous selection model consisting of AST, ALT, Hb and PCV may be effective in minimizing the stress. Abbas *et al.* (2012) have documented that the thermally stressed birds fail to achieve a balance between body heat production and heat loss. HS causes a noticeable effect on the physiological potential of birds (Mashaly *et al.* 2004, Ayo *et al.* 2010). There exists significant ill-effect of HS on health of birds leading to upscaling of CT and alteration in serum biochemical parameters (He *et al.* 2019). With an increase of 1°C body temperature, the metabolic rate of the body increases by 20–30% that scales up heat production leading to surge of the temperature of birds above normal (De Basilio *et al.* 2003, Abbas *et al.* 2008, Zuidhof *et al.* 2010).

The residual effect of other variables on CT during HS was 0.73 and its complement (1-h) takes the value of 0.27. So AST, ALT, Hb and PCV explain only 27% of the variability in CT whereas factors apart from the aforesaid explain only 73% of variability in CT during HS (Singh and Choudhary 1985). This may be attributed to stimulation of all the factors during HS, in both CT and HR (Table 4). Thus a selection model consisting of AST, ALT, Hb and PCV will be effective in controlling the HS in Khadia chicken.

There was a strong correlation ($P<0.01$) between CT and each body part temperature; CT followed a quadratic response to environmental air temperature treatments (Candido *et al.* 2020). During HS condition, the correlation between CT and AST was 0.655 whereas the direct effect of AST on CT was 0.463 (Table 4) indicating both are high in nature which corroborates the finding of previous study. Subjecting to regression analysis, the multiple regression analysis demonstrated HR as dependent variable and AST, ALT, Hb and PCV as independent variable revealing AST and PCV to be critical in selection. Similarly, the multiple regression analysis depicting CT as dependent variable and AST, ALT, Hb and PCV as independent variable revealed that AST is important amongst all these parameters. In the present study, the R^2 value was determined to be 0.7 and 0.49, when HR and CT are dependent variables, respectively. Therefore, considering all these traits, two regression models were formulated which are mentioned below.

$$Y = -202.33 + 2.01X1 - 0.09X2 - 1.89X3 + 4.48X4 \text{ for HR}$$
$$Y = 31.12 + 0.04X1 - 0.0005X2 + 0.09X3 + 0.07X4 \text{ for CT}$$

where Y is the dependent variable heart rate or CT as the case may be

$$X1 = \text{AST},\ X2 = \text{ALT},\ X3 = \text{Hb},\ X4 = \text{PCV}$$

It is concluded that the regression model for HS may be extrapolated for early prediction of stress in native Khadia chicken population. Early determination of the HS traits derived through path analysis would be one of the most promising methods to predict the heat resilient and effective adaptation strategy for rural poultry to the hot and humid conditions. The path coefficient analysis is a step forward to predict HS in chicken which can save time, money and labour as compared to molecular methods.

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