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REGULAR ARTICLES

Use of discriminant analysis for the evaluation of coccidiosis resistance parameters in chickens raised in hot humid tropical environment

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Abstract

Coccidiosis endemicity remains a major challenge in poultry production in the tropics and all over the world. In order to develop predictive tool for identification of chickens that are at risk of coccidiosis among Nigerian indigenous chickens, body weight gain (BWG) and hematological variables were determined for chickens infected with *Eimeria tenella* (female = 60, male = 63) and uninfected (female = 51, male = 45). The hematological variables analyzed include the following: packed cell volume (PCV, %), white blood cells (WBC, $\times 10^6/\mu l$), and red blood cells (RBC, $\times 10^6/\mu l$), as well as differential leucocyte percentages of neutrophils, lymphocytes, monocytes, and eosinophils. Body weight gain was determined at days 3, 6, 9, 12, and 15. Of the 12 variables analyzed, BWG at day 3, monocyte, PCV, and WBC in males and BWG at days 6, 9, and 12, PCV, and WBC in female chickens showed significant ($P \leq 0.01$) difference between the infected and uninfected. Stepwise discriminant analysis evolved a model that could distinguish uninfected from *Eimeria*-infected chickens. Packed cell volume, WBC, BWG at day 3, and lymphocytes emerged the most discriminant between uninfected and *Eimeria*-infected chickens in male chickens. In female chickens, PCV, RBC, and BWG at day 3 were identified as most discriminant variables in separating the uninfected from *Eimeria*-infected chickens. Therefore, this study suggests that routine blood test and estimates of body weight gain could serve as a useful tool for identifying chickens that may be at risk of coccidiosis, enabling improvement of preventive measures.

Keywords Breeding · Coccidiosis · Discriminant analysis · *Eimeria tenella*

Introduction

Coccidiosis is a disease of major economic importance affecting domesticated avian species (Williams 2005). The disease affects birds of all ages. It poses serious public health and economic challenges to both commercial and smallholder poultry farmers globally on a continuous basis (Azeezah et al. 2012). The short direct life cycle and high reproductive potential of coccidians in poultry often leads to severe outbreaks of disease in small backyard flocks or modern poultry house (McDougald and Fitz-Coy 2008). In the tropics, the problem of diseases such as coccidiosis is mostly combated with the use of drugs which invariably add more to the cost of poultry production (Azeezah et al. 2012). Hence, alternatives approach is being sought worldwide as addition to the use of drugs and vaccine. Breeding for resistance to the disease will be one of the possible approaches as addition to the use of drugs and vaccine (Adeleke et al. 2015). Breeding involves

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identification of line, strain, or breed of animal that has potential to resist coccidiosis. Among potential strains of chickens for this genetic selection are Nigerian indigenous chickens. The unique adaptive features of the Nigerian indigenous chickens predisposing it to adapt to the local environment have been reported by several authors (Adebambo et al. 1999; Ikeobi et al. 2001; Peters et al. 2011; Ajayi et al. 2012). These features include disease resistance (Egena et al. 2014), hardiness, and ease of rearing (Ige 2013).

In evaluating coccidiosis resistance characteristics, several variables have been used as coccidiosis resistance variables. These variables include post-inoculation body weight gain, fecal oocyst shedding, and plasma levels of carotenoid, nitrite plus nitrate (Kim et al. 2006; Hong et al. 2009; Kim et al. 2010), and hematological and biochemical variables (Meskerem et al. 2013). Hematological variables have been reported to provide valuable information on the immune status of chickens (Ladokun et al. 2008). This information is useful for diagnostic management purposes as well as breeding programs for the genetic improvement of indigenous chicken. However, evaluation of coccidiosis resistance variables between healthy and infected chickens has been restricted to the use of univariate analysis only. Authors considered the variables individually using univariate analysis. Univariate analysis generally indicates testing for group differences on each of the coccidiosis variables without taking into account its relationships to the other coccidiosis variables. Therefore, in interpreting univariate analysis, caution should be exercised because univariate F tests do not account for correlations among the coccidiosis parameters or any potential increase in Type 1 error (probability of incorrectly rejecting null hypothesis) that results from several univariate analysis being carried out on all the variables (Tenko and George 2008).

Meanwhile, the use of multivariate (discriminant) analysis is considered to be more appropriate. This is due to the joint consideration of all measured coccidiosis parameters at once. Discriminant analysis is where two or more groups are known and one or more new observations are classified into one of the known groups based on the measured characteristics (Asamoah-Boaheng and Sam 2016).

The aims of this study were the following: (1) to use discriminant analysis to study the variability in *Eimeria*-infected and uninfected (normal) chickens, (2) to develop a model which could group the chickens in one of these two groups using only a few of the hematological variables. This is novel approach as to the best of our knowledge; no systematic effort has been reported that attempts to use body weight gain, hematology, and comprehensive statistical tools to find any diagnostic or predictive biomarkers for coccidiosis disease in chickens raised in hot humid tropical environment.

Materials and methods

Study site and experimental materials

The experiment was carried out at the Poultry Breeding Unit of the Directorate of Farm, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. A total of 230 chicks of Nigerian indigenous chickens were obtained through artificial insemination from parent stock kept at the Poultry Breeding Unit. Abeokuta is located within the rainforest zone of Southwestern Nigeria with latitude 7° 13′, 49° 46′ N, longitude 3° 26′, 11° 98′ E and altitude 76 mm above sea. The annual mean temperature and humidity were 34 °C and 82% respectively (Amujoyegbe et al. 2008). The indigenous chickens used in this study were generated from several years of selection of Nigerian local chickens in the Poultry Breeding Unit of the institution. The selection of the chickens started in 1995 when local chickens were sourced from local farmers in Southwest Nigeria. The chickens used in this study were made up of indigenous chickens with the feather distribution gene (Naked Neck), feather structure gene (Frizzle Feather), and normal feathered. More information about this population of chickens has been provided by Peters et al. (2011).

The chicks were brooded for 3 weeks on a commercial chick mash. Feed and water were supplied ad libitum from day-old on a ventilated deep litter system using wood shavings as bedding materials. At 3 weeks of age, the chicks were divided into two groups. In the first group, chicks were inoculated with *Eimeria tenella* (*E. tenella*) which was obtained from National Veterinary Research Institute, Vom, in Plateau State, Nigeria, through oral inoculation at the rate of 1×10^5 doses per chick. All inoculated chicks were raised in battery cages to avoid physical contact with their feces. In the second group, chicks were not inoculated with *E. tenella* and served as control group. Blood samples were collected from the wing web using a 2-ml sterile syringe and needle from each bird from the two groups. Blood samples were collected from both groups 2 weeks after inoculation of the first group into EDTA tube. The total red blood cells were assessed in a 1:200 dilution of blood in Hayem's solution. The differential leukocyte counts were determined by staining blood films with Wright's stain. Packed cell volume was measured using the microhematocrit method. Body weight was taken in the morning before the birds were fed and it was done using a weighing balance scale with sensitivity of 0.01 g. The post-inoculation body weight gain for days 3, 6, 9, 12, and 15 was estimated as $\frac{\text{final body weight-initial body weight}}{\text{number of days}}$.

Data analysis

Incomplete data due to blood clotting from chicks were excluded from the analysis. Preliminary analysis was carried out

where homogeneity was tested. Because of deviation from normality of measurements taken, all data were $\log_{10}$ transformed before analysis. Effects of health status (uninfected or *Eimeria*-infected) on body weight gain and hematological variables in the chickens was determined. Means were separated using Tukey's method. Canonical discriminant analysis was used to identify the combination of coccidiosis parameters that best separate the two groups (uninfected and infected). The combination of measurements that best discriminate between the two groups was selected and a discriminant function model was obtained from there. To identify infected and uninfected chickens, the unstandardized discriminant function procedure was employed (Tenko and George 2008). The ability of this function to identify infected chickens with *Eimeria tenella* from uninfected chickens was indicated as the percentage of individuals correctly classified from the samples that generated the function. Accuracy of the classification was evaluated using a priori method at $p \leq 0.05$. All analyses were done using SAS (2010).

Results and discussion

The mean (± SE) values of the coccidiosis parameters presented in Table 1 showed significant differences ($P < 0.01$) in body weight gain at day 3 (BWG 3), monocyte (Mono), packed cell volume (PCV), and white blood cells (WBC) in male chickens. Only PCV and WBC as well as BWG 3, 6, and 12 were significantly different in female chickens. Reduction observed in PCV of infected male and female chickens was comparable to those observed by Fukata et al. (1997) and Meskerem et al. (2013) who reported lower counts of red blood cells and PCV in chickens infected with *Eimeria tenella* when they were compared to the uninfected chickens. Increase in WBC count obtained in this study was similar to results of Ricklefs and Sheldon (2007), who reported high counts of WBC in infected animals.

Although the univariate statistics showed significant differences in some variables, the multivariate method provided better resolution. Wilk's lambda was used for multivariate

Table 1 Means and standard errors of body weight gain, fecal egg count, lesion scores, and hematological variables in the *Eimeria*-infected and non-infected Nigerian indigenous chickens

Variable	Chickens' grouping	Male chickens		Female chickens	
		Mean ± SE	*N*	Mean ± SE	*N*
Weight gain at day 3	Infected	0.8966 ± 0.0448^{b}	63	0.7514 ± 0.0606	60
	Uninfected	0.9839 ± 0.0527^{a}	45	0.8309 ± 0.0633	51
Weight gain at day 6	Infected	0.8808 ± 0.0623	63	0.6027 ± 0.0361^{b}	60
	Uninfected	0.9300 ± 0.0369	45	0.8779 ± 0.0540^{a}	51
Weight gain at day 9	Infected	0.9460 ± 0.0495	63	0.7892 ± 0.0390^{b}	60
	Uninfected	1.0441 ± 0.0374	45	1.0133 ± 0.0555^{a}	51
Weight gain at day 12	Infected	0.8204 ± 0.0551	63	0.7891 ± 0.0486^{b}	60
	Uninfected	0.8993 ± 0.0579	45	0.8764 ± 0.0408^{a}	51
Weight gain at day 15	Infected	0.7498 ± 0.0560	63	0.6595 ± 0.0582	60
	Uninfected	0.7853 ± 0.0597	45	0.7591 ± 0.0604	51
Eosinophils (%)	Infected	0.2852 ± 0.0513	63	0.2397 ± 0.0379	60
	Uninfected	0.5129 ± 0.0364	45	0.2016 ± 0.0397	51
Lymphocyte (%)	Infected	1.8241 ± 0.0099	63	1.8279 ± 0.0077	60
	Uninfected	1.8251 ± 0.0098	45	1.8102 ± 0.0092	51
Monocyte (%)	Infected	0.6183 ± 0.0514^{a}	63	0.4851 ± 0.0377	60
	Uninfected	0.4446 ± 0.0374^{b}	45	0.4369 ± 0.0463	51
Neutrophils (%)	Infected	1.4865 ± 0.0197	63	1.5019 ± 0.0197	60
	Uninfected	1.6005 ± 0.0212	45	1.4768 ± 0.0226	51
Packed cell volume (%)	Infected	1.3695 ± 0.0095^{b}	63	1.3565 ± 0.0141^{b}	60
	Uninfected	1.4440 ± 0.0099^{a}	45	1.4903 ± 0.0139^{a}	51
Red blood cells ($\times 10^3 \mu l$)	Infected	0.7602 ± 0.0199	63	0.7528 ± 0.0115	60
	Uninfected	0.7776 ± 0.0194	45	0.7629 ± 0.0186	51
White blood cells ($\times 10^3 \mu l$)	Infected	0.9105 ± 0.0331^{a}	63	0.9059 ± 0.0329^{a}	60
	Uninfected	0.7009 ± 0.0440^{b}	45	0.7872 ± 0.0519^{b}	51

Means in the same row bearing different superscripts are significantly different ($P < 0.01$)

SE standard error, *N* sample size

Table 2 Weight gain, FEC, lesion scores, and hematological variables pain the *Eimeria*-infected and non-infected Nigerian indigenous chickens

Variable	Male chickens			Variable	Female chickens		
	Wilks' lambda	*P* level	Tolerance		Wilks' lambda	*P* level	Tolerance
Packed cell volume	0.7210	0.0001	1.0000	Packed cell volume	0.6448	0.0001	1.0000
White blood cells	0.5475	0.0001	0.9993	Weight gain at day 3	0.3636	0.0001	0.9025
Weight gain at day 3	0.4719	0.0001	0.9939	Red blood cells	0.3482	0.0001	0.8493
Lymphocyte	0.4547	0.0001	0.8516				

statistical test of group differences in male and female chickens (Table 2). The two groups do differ significantly when the coccidiosis resistance variables are considered simultaneously. This implies significant discriminant function (a linear combination of the parameters). This was evidenced by the selection of four variables (PCV, WBC, BWG 3, and lymphocytes) in male chickens and three variables (PCV, RBC, and BWG 3) out of the 12 variables assigned for stepwise discriminant analysis. A stepwise discriminant analysis was carried out to determine if any of the variables could be used to categorize chickens into one of the two groups (*Eimeria*-infected and uninfected chickens). The results implied that PCV, WBC, BWG 3, and lymphocytes were observed to be the most informative variables to effectively place infected and uninfected chickens in distinct group for male chickens, while in female chickens, PCV, RBC, and BWG 3 were the main variables in differentiating between infected and uninfected chickens. PCV and BWG 3 were found to be important variables in distinguishing infected and uninfected chickens in both sexes. This implied that factors leading to coccidiosis may also cause significant changes in other physiological characteristics such as hematology and body weight gain which may be used as substitute signs for coccidiosis disease. Data from the stepwise discriminant analysis was successively used to develop linear models representing the contribution of each of the important variables to be able to distinguish between the infected and uninfected groups. The discriminating variables extracted for each sex were included in a discriminant equation (Y):

$$Y = -742.46 + 389.26PCV - 14.77WBC - 0.62BWG3 + 527.17LYMP \quad \text{Male}$$
$$Y = -140.35 + 162.609PCV + 55.62RBC + 20.50BWG3 \quad \text{Female}$$

With the discriminant equation, new measurements of PCV, RBC, and BWG 3 could be assigned into the equation to estimate discriminant scores using a pocket calculator. Positive discriminant score indicates the chicken is infected while negative discriminant score indicates uninfected chicken. A similar study was carried out by Alshamisi et al. (2013) in camels using only hematological parameters to distinguish fracture, lame, and normal camels.

The effectiveness of the model to be able to discriminate between the two chicken groups using coccidiosis variables in each of the model was also tested. The discriminant function was able to correctly classify 90.48% of the 63 infected chickens and 77.78% of the 45 uninfected chickens in male

Table 3 Classification of Nigerian indigenous chickens based on the discriminant model for males and females

Sex		Animal group	Predicted group membership		
			Infected	Uninfected	Total
Male	Original count	Infected	57	6	63
		Uninfected	10	35	45
	%	Infected	90.48	9.52	100
		Uninfected	22.22	77.78	100
Female	Original count	Infected	59	1	60
		Uninfected	12	39	51
	%	Infected	98.33	1.67	100
		Uninfected	23.53	76.47	100
	Cross-validated count	Infected	63	45	108
		Uninfected	60	51	111
	%	Infected	58.33	41.67	100
		Uninfected	54.05	45.95	100

chickens, while in female, 98.33% of the 60 infected and 76.47% of the 51 uninfected chickens were studied (Table 3). Female infected chickens were more accurately differentiated than their male counterpart. Cross validation with the prior method indicated 85.19% of the original grouped correctly classified in male while 88.29% of the original group correctly classified in female. This study's finding is relevant in order to establish measurable evaluation model for *E. tenella* resistance and to explore new coccidiosis resistance breeding method for chickens.

Conclusion

Statistical models developed in this study as predictive tool could successfully differentiate infected from uninfected chickens using results from routine hematological tests. In both males and female chickens, over 90% of infected chickens could be successfully distinguished from the uninfected chicken group using four variables (PCV, WBC, LYMP, and BWG 3) in males and three variables (PCV, RBC, and BWG 3) in female chickens. We therefore conclude that in diagnosing and predicting chicken infected with *Eimeria* our two models could be used with minimum rate of misclassification without involving all clinical signs, biochemical, pathmorphological and histological analyses.

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Compliance with ethical standards

The manuscript does not contain clinical studies or patient data.

Statement of animal rights The Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria, approved all the procedures used for the research.

Conflict of interest The authors declare that they have no conflict of interest.

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