Tropical Animal Health and Production (2021) 53: 385
https://doi.org/10.1007/s11250-021-02841-w

REGULAR ARTICLES

Discriminant analysis of response to Newcastle disease and heat tolerance among chicken genotypes in hot humid tropical environment

S. O. Durosaro[1] · B. M. Ilori[1] · D. O. Oguntade[2] · O. S. Iyasere[3] · A. O. Adebambo[1] · M. O. Ozoje[1]

Received: 23 March 2021 / Accepted: 24 June 2021 / Published online: 2 July 2021

Abstract
Newcastle disease and heat stress reduce the productivity of local chickens of Nigeria (LCN). This study compared the antibody response to Newcastle disease and heat tolerance among different LCN genotypes in hot humid tropics using multivariate discriminant analysis. A total of 299 birds were used for the study. Geometric mean titre against Newcastle disease before vaccination (GMTB), geometric mean titre against Newcastle disease after vaccination (GMTA), rectal temperature at week 4 (RT4), pulse rate at week 4 (PR4), respiratory rate at week 4 (RR4), heat stress index at week 4 (H4), rectal temperature at week 13 (RT13), pulse rate at week 13 (PR13), respiratory rate at week 13 (RR13) and heat stress index at week 13 (H13) were measured. All the traits were significantly ($p < 0.05$) affected by the genotype while sex differences were only observed in GMTB, GMTA and RR13. The stepwise discriminant analysis revealed RR4, PR13, RT13, H4, GMTA, GMTB, H13 and RT4 to be effective in differentiating the three chicken genotypes. Two canonical variables that accounted for 60.21% and 39.79% of the total variation were revealed. Linear discriminant functions for differentiation of the three chicken genotypes were also developed. 87.39% of normal feather, 76.58% of naked neck and 100% of frizzle feather chickens were correctly assigned into their genotypes. The longest Mahalanobis distance was observed between normal feather and frizzle feather chickens. The discriminant functions developed in this study could be used to differentiate the three genotypes of LCN using antibody response to Newcastle disease and heat tolerance.

Keywords Antibody response · Discriminant analysis · Heat stress index · Pulse rate · Rectal temperature · Respiratory rate

Introduction

The role of native poultry in the diet of man is very essential in all countries, particularly in developing countries where the bulk of the populace reside in rural parts and survive on village birds as the main supply of animal protein (Padhi 2016). For instance, the Nigerian poultry industry comprises about 180 millions birds. About 83 million of the birds are raised in free-range system, 59 million in semi-intensive system and the remaining 38 million birds are raised in intensive system (FAO 2019).

The local chickens of Nigeria (LCN) are mainly reared for meat and egg production (Manyelo et al. 2020). They are of three genotypes which are normal feather, naked neck and frizzle feather. They are found in all parts of Nigeria and represent a considerable part of the Nigerian rural wealth and also of the national economy (Wong et al. 2017). They are self-reliant and can survive on little inputs (Egahi et al. 2010).

The hot environment where LCN are found is characterized with high rate of infectious disease (Polgreen and Polgreen 2018) and harsh weather conditions. Newcastle disease is one of the diseases that limit the productivity of LCN and this may be due to the fact that the causative organism of Newcastle disease can survive for many days in a hot and wet environment, on birds' feathers and droppings (Alexander 2012). It is a key viral disease of serious economic significance in chickens (Wulan et al. 2017)

✉ S. O. Durosaro
durosaroso@funaab.edu.ng

[1] Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

[2] Department of Animal Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

[3] Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

and one of the utmost disease constraints to the growth of backyard chicken production in tropical regions (Ashraf and Shah 2014). Lethal strains of Newcastle disease virus can wipe off chicken flocks with or without clinical symptoms particularly in unvaccinated chickens. The disease has also been reported in vaccinated chickens, probably due to vaccine failure (Shittu et al. 2016). Hundred percent prevalence of Newcastle disease was reported by Bobbo et al. (2013) in Adamawa State, Nigeria, for normal feather, naked neck and frizzle feather Nigerian local chickens, respectively. Unigwe et al. (2020) reported 13.56% prevalence of Newcastle disease in normal feather Nigerian local chickens raised in Ibadan, Oyo State, Nigeria. Balami et al. (2014) reported 36.7% prevalence of Newcastle disease in local chickens raised in Borno State, Nigeria; Wakawa et al. (2014) reported 29.1% Newcastle disease prevalence in Nigerian local chickens raised in Kaduna State of Nigeria while Lawal et al. (2015) reported 55.5% prevalence in local chickens raised in Gombe State, Nigeria.

Aside Newcastle disease prevalence in the tropical environments such as Nigeria, heat stress from high temperature and humidity is also a risk to the maximization of high potential of LCN. Heat stress is one of the key factors affecting intake, weight gain, mortality rate and profitability of livestock species (Idris et al. 2021). A rise in the respiratory rate is the primary observable sign that chickens make in reaction to heat stress. Changes in rectal temperature, heart rate also known as pulse rate and respiratory rate are used as physiological indices of heat stress (Adedeji et al. 2015). These physiological indices are easy to assess, cheap to measure and less invasive.

The present study aimed to compare the antibody response to Newcastle disease and heat tolerance among the different LCN genotypes raised in hot humid tropics using multivariate discriminant analysis. The information obtained from this study will ensure better characterization of LCN raised in hot humid tropics, which will aid further selection, conservation and genetic improvement of the birds. To the best of our knowledge, the present study is novel as the earlier reports on discriminant analysis in LCN were based on morphological traits.

Materials and methods

Experimental site

The chickens were raised at the Poultry Breeding Unit of the University Farms, Federal University of Agriculture, Abeokuta, Alabata, Ogun State, Nigeria. Alabata (latitude 7° 10′ N and longitude 3° 2′ E) is located in Odeda Local Government Area of Ogun State, Nigeria (Google Map 2021). The area is a hot humid tropical environment. It lies in the South Western part of Nigeria and has a mean annual rainfall of about 1037 mm. The mean ambient temperature of the area ranges from 28 °C in December to 36 °C in February. The area has a yearly average relative humidity of about 82% (Climate data 2021).

Experimental birds and management

Two hundred and ninety-nine (299) LCN were used for the experiment. The birds comprised 111 normal feather (52 males and 59 females), 111 naked neck (76 males and 35 females) and 77 frizzle feather (45 males and 32 females). All the birds were raised together in a pen measuring 30 m × 25 m under intensive management system. The birds were wing-tagged for proper identification. They were all subjected to the same management procedures. Chick starter mash containing 23% crude protein and 11.1 MJ/kg metabolizable energy was fed to the birds *ad libitum* from 0 to 8 weeks of age. Grower mash containing 18% crude protein and 10.48 MJ/kg metabolizable energy was fed to the birds *ad libitum* from 9 to 13 weeks of age. Clean water was also provided for the birds without restriction. Marek's disease vaccine was administered at day 1. Infectious bursal disease vaccine was administered at day 21 while fowl pox disease vaccine was administered at day 70. The ethical protocol for the research was approved by Animal Care and Use Committee of Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Vaccination with Newcastle disease vaccine procedure

The LCN were vaccinated with a commercial Newcastle disease virus vaccine (LaSota strain), using the standard dose at 25 and 50 days of age, respectively.

Blood collection and serum preparation

One millilitre (1 mL) of blood was collected from brachial vein of each LCN with needle and syringe at day 24 (about 4th week of age) pre-vaccination with Newcastle disease vaccine. Blood was also collected from birds at 41 days after second vaccination (91 days of age, equivalent to 13th week of age). Serum was prepared from the blood samples collected pre-vaccination and post-vaccination by centrifuging at 5000 revolution per minute for 5 min in Thermo Scientific refrigerated centrifuge at 4 °C.

Determination of baseline antibody response to Newcastle disease virus

Serum prepared from blood of unvaccinated LCN was used to determine the baseline antibody response to Newcastle

disease virus, henceforth referred to as basal geometric mean titre (GMTB). The baseline antibody response to Newcastle disease virus was determined by haemagglutination inhibition test according to Allan and Gough (1974). Briefly, 25 µl of phosphate-buffered saline was added to all the wells of a microtitre plate and 25 µl of the serum of the LCN was added to the first well and serially diluted. Twenty-five microlitre (25 µl) of the diluted antigen was added to all the wells. Finally, 25 µl of 1% chicken red blood cell was added to all the wells and the microtitre plate incubated at room temperature for 30 min. The antibody response was measured as the reciprocal dilutions where there was complete inhibition of the agglutination of the chicken red blood cell. The obtained reciprocal of the first dilution was 2.0.

Determination of antibody response to Newcastle disease virus after vaccination

Serum prepared from blood collected at 41 days after second vaccination was used to determine antibody response post vaccination, henceforth referred to as geometric mean titre after vaccination (GMTA). This was also determined according to Allan and Gough (1974).

The end point for each LCN sample (both pre-vaccination and post-vaccination) was converted to geometric mean titre (GMT) using the formula (Villegas and Purchase 1989):

$$\mathrm{GMT} = \mathrm{Antilog}\left[(\mathrm{A}-1)\times \mathrm{logB} + \mathrm{logC}\right]$$

where A is the average end point well number, B is factor 2.0 and C is the reciprocal of the first dilution (2.0).

Heat tolerance traits

Rectal temperature, pulse rate and respiratory rate of LCN were recorded in the morning between 7:00 am and 8:00 am and again between 2:00 pm and 3:00 pm of the same day. The heat tolerance traits were recorded at weeks 4 and 13 corresponding to the time when antibody responses to Newcastle disease virus were determined. Rectal temperature was measured by inserting a digital rectal thermometer into the rectum of the bird and recorded after the sound of the alarm signal. Respiratory rate was determined by counting the number of movement of the vent per minute. Pulse rate was recorded as number of movement of the brachial vein per minute. Heat stress index was calculated using the formula (Oladimeji et al. 1996):

$$\mathrm{H} = (\mathrm{AR}/\mathrm{AP}) * (\mathrm{NP}/\mathrm{NR})$$

where H is the heat stress index, AR is the average respiratory rate of chicken, AP is the average pulse rate of chicken, NP is the normal pulse rate of chicken and NR is the normal respiratory rate of chicken. 290 beats/min and 32 breaths/min were used as normal pulse and respiratory rates, respectively, as reported by Dagon (1989). Chickens with H value of ≥ 4.50 are considered stressed.

Statistical analysis

The antibody response to Newcastle disease virus was transformed to geometric mean titre for data normalization. Genotype and sex effects on antibody response to Newcastle disease and heat tolerance were determined using GLM procedure while means were separated using Duncan's multiple range test at 95% confidence level. STEPDISC procedure was used to determine the variables that contributed to differentiation among the three genotypes of LCN. CANDISC procedure was used to obtain canonical variables, canonical coefficients and Mahalanobis distance among the three genotypes of LCN based on the selected traits. The DISCRIM procedure was used to find the percentage of correct assignment of each bird to its genotype. All analyses were performed using SAS version 9 (2002). Geometric mean titre against Newcastle disease before vaccination, geometric mean titre against Newcastle disease after vaccination, rectal temperature at week 4, pulse rate at week 4, respiratory rate at week 4, heat stress index at week 4, rectal temperature at week 13, pulse rate at week 13, respiratory rate at week 13 and heat stress index at week 13 were the ten variables used for the analysis.

Results

The means and standard errors of the antibody response to Newcastle disease and heat tolerance traits according to genotype, sex and genotype by sex interaction are shown in Table 1. All the 10 traits were significantly ($p < 0.05$) affected by the genotype. The highest ($p < 0.05$) GMTB was observed in naked neck chickens while there was no difference in GMTB of normal feather and frizzle feather chickens. The lowest ($p < 0.05$) respiratory rate at week 4 (RR4) was observed in frizzle feather chickens while the highest ($p < 0.05$) respiratory rate at week 13 (RR13) was observed in normal feather chickens. Generally, lower respiratory rates were observed in LCN genotypes and the two sexes at week 4 compared with week 13. Sex differences were only observed in GMTB, GMTA and RR13 with females having higher ($p < 0.05$) GMTB and GMTA. Only the GMTB, pulse rate at week 4, heat stress index at week 4 and pulse rate at week 13 were significantly ($p < 0.05$) affected by the genotype by sex interaction. The highest ($p < 0.05$) GMTB was observed in female naked neck chickens while the lowest ($p < 0.05$) pulse rate at week 4 was observed in male normal feather chickens.

Table 1 Means and standard errors of antibody response to Newcastle disease and heat tolerance traits of local chicken of Nigeria according to genotype, sex and genotype by sex interaction

Trait	Genotype			Sex		Genotype by sex interaction					
	Normal feather ($n=111$)	Naked neck ($n=111$)	Frizzle feather ($n=77$)	Male ($n=173$)	Female ($n=126$)	Male normal feather ($n=52$)	Female normal feather ($n=59$)	Male naked neck ($n=76$)	Female naked neck ($n=35$)	Male frizzle feather ($n=45$)	Female frizzle feather ($n=32$)
GMTB	1.26 ± 0.06^{b}	4.91 ± 1.69^{a}	1.00 ± 0.00^{b}	1.00 ± 0.00^{b}	4.68 ± 1.49^{a}	1.00 ± 0.00^{b}	1.49 ± 0.11^{b}	1.00 ± 0.00^{b}	13.40 ± 5.13^{a}	1.00 ± 0.00^{b}	1.00 ± 0.00^{b}
GMTA	36.77 ± 3.97^{b}	75.35 ± 9.73^{a}	56.13 ± 6.84^{ab}	42.35 ± 5.92^{b}	74.92 ± 6.10^{a}	16.02 ± 2.33	55.05 ± 6.31	70.74 ± 12.53	85.37 ± 14.69	24.84 ± 3.33	100.13 ± 12.14
RT4	41.43 ± 0.05^{a}	41.15 ± 0.05^{b}	41.34 ± 0.03^{a}	41.29 ± 0.04	41.33 ± 0.04	41.45 ± 0.07	41.42 ± 0.06	41.15 ± 0.06	41.15 ± 0.08	41.34 ± 0.07	41.35 ± 0.09
PR4	184.41 ± 1.90^{b}	206.42 ± 2.81^{a}	205.47 ± 3.62^{a}	198.74 ± 2.36	196.99 ± 2.32	177.52 ± 3.72^{c}	190.48 ± 3.50^{b}	208.41 ± 3.08^{a}	202.11 ± 4.54^{a}	206.93 ± 4.00^{a}	203.41 ± 4.75^{a}
RR4	72.44 ± 1.07^{b}	76.49 ± 1.23^{a}	46.35 ± 0.40^{c}	67.72 ± 1.33	66.55 ± 1.31	73.89 ± 1.47	71.17 ± 1.38	76.25 ± 1.22	77.00 ± 1.79	46.18 ± 1.58	46.59 ± 1.88
H4	3.60 ± 0.06^{a}	3.45 ± 0.08^{a}	2.11 ± 0.05^{b}	3.19 ± 0.08	3.12 ± 0.07	3.81 ± 0.10^{a}	3.42 ± 0.09^{b}	3.42 ± 0.08^{b}	3.51 ± 0.12^{b}	2.09 ± 0.10^{c}	2.14 ± 0.12^{c}
RT13	41.20 ± 0.04^{b}	41.65 ± 0.03^{a}	41.64 ± 0.03^{a}	41.54 ± 0.03	41.40 ± 0.03	41.21 ± 0.04	41.19 ± 0.04	41.69 ± 0.04	41.57 ± 0.05	41.66 ± 0.05	41.61 ± 0.06
PR13	171.15 ± 2.05^{b}	211.64 ± 2.97^{a}	205.58 ± 2.88^{a}	202.69 ± 2.44^{a}	184.56 ± 2.63^{b}	173.29 ± 3.56^{c}	169.27 ± 3.34^{c}	218.87 ± 2.94^{a}	195.94 ± 4.34^{b}	209.33 ± 3.82^{b}	200.31 ± 4.54^{b}
RR13	37.04 ± 0.62^{a}	34.68 ± 0.57^{b}	33.30 ± 0.87^{b}	35.34 ± 0.57	35.01 ± 0.52	37.81 ± 0.93	36.36 ± 0.87	34.80 ± 0.77	34.40 ± 1.13	33.38 ± 0.10	33.19 ± 1.18
H13	2.00 ± 0.05^{a}	1.52 ± 0.04^{b}	1.49 ± 0.04^{b}	1.63 ± 0.04	1.77 ± 0.04	2.02 ± 0.06	1.99 ± 0.05	1.47 ± 0.05	1.63 ± 0.07	1.46 ± 0.06	1.53 ± 0.07

GMTB, geometric mean titre against Newcastle disease before vaccination; *GMTA*, geometric mean titre against Newcastle disease after vaccination; *RT4*, rectal temperature at week 4; *PR4*, pulse rate at week 4; *RR4*, respiratory rate at week 4; *H4*, heat stress index at week 4; *RT13*, rectal temperature at week 13; *PR13*, pulse rate at week 13; *RR13*, respiratory rate at week 13; *H13*, heat stress index at week 13

[abc]Means in the same row with different superscripts differ significantly ($p < 0.05$) among the three genotypes as well as genotype by sex interaction

[ab]Means in the same row with different superscripts differ significantly ($p < 0.05$) between the two sexes

Table 2 Traits selected by stepwise discriminant analysis to differentiate among the chicken genotypes

Variable entered	Partial R^2	F-value	Pr > F	Wilks' Lambda	Pr < Lambda	Average squared canonical correlation	Pr > ASCC
RR4	0.581	205.23	< 0.0001	0.419	< 0.0001	0.291	< 0.0001
PR13	0.324	70.62	< 0.0001	0.283	< 0.0001	0.452	< 0.0001
RT13	0.147	25.24	< 0.0001	0.242	< 0.0001	0.500	< 0.0001
H4	0.092	14.92	< 0.0001	0.220	< 0.0001	0.527	< 0.0001
GMTA	0.052	7.93	0.0004	0.208	< 0.0001	0.540	< 0.0001
GMTB	0.030	4.49	0.0120	0.202	< 0.0001	0.547	< 0.0001
H13	0.033	4.87	0.0083	0.195	< 0.0001	0.555	< 0.0001
RT4	0.017	2.51	0.0831	0.192	< 0.0001	0.559	< 0.0001

RR4, respiratory rate at week 4; *PR13*, pulse rate at week 13; *RT13*, rectal temperature at week 13; *H4*, stress index at week 4; *GMTA*, geometric mean titre against Newcastle disease after vaccination; *GMTB*, geometric mean titre against Newcastle disease before vaccination; *H13*, heat stress index at week 13; *RT4*, rectal temperature at week 4

Table 3 Multivariate statistics and F approximations for testing the significance of canonical correlations between traits and genotypes

Statistics	Value	F-value	Num Df	Den Df	Pr > F
Wilks' lambda	0.192	46.31	16	578.00	< 0.0001
Pillai's trace	1.118	45.92	16	580.00	< 0.0001
Hotelling-Lawley trace	2.595	46.74	16	469.34	< 0.0001
Roy's greatest root	1.562	56.64	8	290.00	< 0.0001

Eight out of the original ten traits were effective in differentiating the three chicken genotypes. RR4 was the most discriminating variable followed by pulse rate at week 13 (PR13), rectal temperature at week 13 (RT13), heat stress index at week 4 (H4), GMTA, GMTB, heat stress index at week 13 (H13) and rectal temperature at week 4 (RT4), respectively (Table 2).

The multivariate statistics and F approximations for testing the significance of canonical correlations between traits and genotypes are shown in Table 3. The Pr > F values of all Box's M statistics were highly significant, indicating that the data did not differ significantly from multivariate normal and we could proceed with the analysis.

The canonical analysis carried out on antibody response to Newcastle diseases and heat tolerance traits identified two significant ($P < 0.05$) canonical variables that accounted for 60.210% and 39.790% of the total variation. Generally, any variable with loading of 0.30 (or higher) is considered to contribute significantly as a discriminating variable. RR4 and H4 were highly correlated with CAN 1 while PR13, RT13 and H13 were highly correlated with CAN 2. Variable GMTA was moderately correlated with CAN 2 while H13 was moderately correlated with CAN 1. Canonical correlations of 0.781 and 0.713 were obtained for CAN 1 and CAN 2, respectively (Table 4).

Table 4 Total canonical structure of the three chicken genotypes

Variable	CAN 1	CAN 2
RR4	0.943	0.278
PR13	−0.330	0.722
RT13	−0.387	0.671
H4	0.858	0.015
GMTA	−0.035	0.306
GMTB	0.083	0.214
H13	0.387	−0.558
RT4	−0.015	−0.347
Canonical correlation	0.781	0.713
Eigen value	1.562	1.032
Approximate standard error	0.023	0.029
Variance accounted for (%)	60.210	39.790
Cumulative variance (%)	60.210	100.000

Variables retained by stepwise discriminant analysis were used to develop linear models representing the contribution of each of the selected traits in order to differentiate the three chicken genotypes. The linear discriminant functions for the three chicken genotypes are shown in Table 5.

87.39% of normal feather, 76.58% of naked neck and 100% of frizzle feather chickens (an average of 87.99% for the three genotypes) were correctly assigned into their genotypes (Table 6).

The Mahalanobis distance among the three chicken genotypes is shown in Table 7. The longest Mahalanobis distance was observed between normal feather and frizzle feather chickens while the shortest was observed between normal feather and naked neck chickens.

Table 5 Linear discriminant functions for the three chicken genotypes

Genotype	Linear discriminant function
Normal feather	Y = − 12,325 + 3.505RR4 − 1.256PR13 + 439.326RT13 − 52.953H4 + 0.167GMTA + 0.059GMTB − 49.877H13 + 163.985RT4
Naked neck	Y = − 12,482 + 3.644RR4 − 1.225PR13 + 443.779RT13 − 54.839H4 + 0.176GMTA + 0.116GMTB − 51.181H13 + 163.125RT4
Frizzle feather	Y = − 12,479 + 3.260RR4 − 1.241PR13 + 443.539RT13 − 52.746H4 + 0.178GMTA + 0.091GMTB − 51.992H13 + 163.840RT4

Table 6 Percentage (%) of individual chicken classified into three chicken genotypes

Genotype	Normal feather	Naked neck	Frizzle feather
Normal feather	87.39	3.60	9.01
Naked neck	16.22	76.58	7.21
Frizzle feather	0.00	0.00	100
Priors	0.33	0.33	0.33
Error rate	0.13	0.23	0.00

Table 7 Mahalanobis distance among the three chicken genotypes

	Normal feather	Naked neck	Frizzle feather
Normal feather	0		
Naked neck	37.58***	0	
Frizzle feather	54.25***	50.48***	0

***P < 0.001

Discussion

The dissimilarity in the GMTB of LCN may be attributed to partial development of their immune system at 24 day of age (Bello et al. 2018) and variation among the three genotypes in their maternal effect (Deka et al. 2020). The highest GMTB generated by naked neck chickens might be due to high maternally derived antibody transmitted from hens to chick which protects them when young. The lowest GMTB in frizzle feather chicken observed in this study differed from the findings of Ikpeme et al. (2019) who found the highest pre-vaccination immune response to Newcastle disease in frizzle feather chicken. Sex differences observed in GMTB was expected as Osei-Amponsah et al. (2013) also reported sex differences in immunocompetence in Ghanaian local, Sasso T-44 and broiler chickens.

Genotype markedly influenced the bird's physiological response to heat. The different heat responses might have resulted from thermoregulatory roles of feather distribution and feather structure genes. Heat stress index measures the deviation of the observed respiratory rate and pulse rate from normal values. The more the heat stress index, the more the severity of the heat stress. The value of heat stress index obtained in this study implied that the normal feather chickens were more thermally stressed at week 13 than other genotypes.

Selection of traits that can discriminate the three chicken genotypes is a crucial step towards selection, improvement and conservation of LCN (Varga et al. 2020). Heat tolerance traits and antibody response to Newcastle disease before and after vaccination can be used to differentiate LCN genotypes. A high genetic dissimilarity was observed among the three chicken genotypes. A distinct differentiation of the three genotypes was created by the two canonical variables observed in this study. Ogah (2013) also obtained two canonical variables in description of morphological traits of three genotypes of LCN. Out of all the selected variables used to differentiate the three chicken genotypes, RR4, PR13, RT13 and H4 made the highest contribution to the discriminant function while GMTA, H13 and RT4 made moderate contribution to the function as any variable with loading of 0.30 or more has been reported by Adeyemi and Oseni (2018) to contribute significantly to a discriminant function. Our results on discriminant analysis of LCN using antibody response to Newcastle disease and heat tolerance traits provided validity for the analysis. The CAN 1 and CAN 2 explained 60.21% and 39.79% of the total variation, respectively. This implies that CAN 1 has the best linear combination of antibody response to Newcastle disease and heat tolerance traits enough in discriminating the three chicken genotypes.

Using the discriminant functions derived for each genotype of LCN, new measurements of RR4, PR13, RT13, H4, GMTA, H13 and RT4 can be inserted into the discriminant functions to estimate discriminant scores as suggested by Adenaike et al. (2018). The accuracy of the discriminant functions to discriminate the three chicken genotypes using antibody response to Newcastle disease and heat tolerance traits was also checked with discriminant function of the frizzle feather chickens having 100% reliability.

Mahalanobis distance established significant differentiation among the three chicken genotypes. Frizzle feather chickens were more separated from the other two genotypes. The distance revealed by the Mahalanobis test is an indication that the three chicken genotypes respond differently to Newcastle disease and also tolerate heat differently. This might be linked to different feather distribution and structure of the birds. The feather distribution gene, naked gene (*Na*), and the feather structure gene, frizzle (*F*) gene, are

economically important in chicken breeding systems as they are involved in disease resistance and heat tolerance. These two genes cause reduction in tropical thermal stress by improving the breed's ability for convection (Oke 2011). The naked neck gene has also been related with heat tolerance as Cahaner et al. (2008) reported that heat resistance correlates with reduced feather density.

The present findings are novel as the earlier reports on discriminant analysis in LCN were based on majorly on morphological traits.

Conclusions

Genotypic dissimilarity existed in antibody response to Newcastle disease and heat tolerance in LCN raised in hot humid tropics. Sex differences also existed in response to Newcastle disease in LCN. The RR4, PR13, RT13 and H4 were found as the highly discriminating variables while GMTA, H13 and RT4 are moderately discriminating variables in genotypes of LCN. The discriminant functions developed in this study could be used to differentiate the three genotypes of LCN using antibody response to Newcastle disease and heat tolerance.

Author contribution S.O. Durosaro was involved in conceptualization, investigation, data collection, data analysis and manuscript writing.

B.M. Ilori was involved in data collection, data analysis and manuscript writing.

D.O. Oguntade was involved in investigation and manuscript writing.

O.S. Iyasere was involved in investigation, data collection and manuscript writing.

A.O. Adebambo was involved in investigation, data collection, data analysis and manuscript writing.

M.O. Ozoje was involved in investigation, data collection and manuscript writing.

Funding This work was funded by Africa Centre of Excellence in Agricultural Development and Sustainable environment, Federal University of Agriculture, Abeokuta, Nigeria (Grant Number: ACE023).

Availability of data and material The data are available upon request.

Code availability The codes for analyses are available upon request.

Declarations

Ethics approval The protocol for the experiment was approved by Animal Care and Use Committee of College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Adedeji, T.A., Amao, S.R., Ogundairo, O.M. & Fasoyin O.A. 2015. Heat tolerance attributes of Nigerian locally adapted chickens as affected by strain and some qualitative traits. Journal of Biology, Agriculture and Healthcare, 5(17), 50-55.

Adenaike, A.S., Peters, S.O., Adeleke, M.A., Fafiolu, A.O., Takeet, M.I. & Ikeobi, C.O.N. 2018. Use of discriminant analysis for the evaluation of coccidiosis resistance parameters in chickens raised in hot humid tropical environment. Tropical Animal Health and Production, 50(5), 1161-1166.

Adeyemi, M.A. & Oseni, S.O. 2018. Canonical discriminant analysis applied to biometric data of Nigerian indigenous turkeys. Archivos de Zootecnia, 67(257), 7-12

Alexander, D.J. 2012. The long view: A selective review of 40 years of Newcastle disease research. Avian Pathology, 41, 329-335.

Allan, W.H. & Gough, R.E. 1974. Standard haemagglutination inhibition test for the Newcastle disease: Vaccination and challenge. Veterinary Record, 95, 147-149.

Ashraf, A. & Shah, M.S. 2014. Newcastle disease: Present status and future challenges for developing countries. African Journal of Microbiology Research, 8(5), 411-416.

Balami, A.G., Ndahi, J., Zaifada, A., Mustapha, M., Jarafu, D. & Asogwa, N. 2014. A retrospective study of poultry diseases diagnosed in Maiduguri, North East, Nigeria. Poultry Fish Wildlife Science, 2, 113.

Bello, M.B., Yusoff, K.M., Ideris, A., Bejo, M.H., Peeters, B.P.H., Jibril, A.H., Tambuwal, F.M. & Omar A.R. 2018. Genotype diversity of Newcastle disease virus in Nigeria: Disease control challenges and future outlook. Advances in Virology, 2018, 6097291.

Bobbo, A.G., Baba S.S., Yahaya, M.S. & El-yuguda, A.D. 2013. Susceptibility of Three Phenotypes of Village Chickens to Newcastle Disease in Adamawa State. Alexandria Journal of Veterinary Sciences, 39, 133-140.

Cahaner, A., Ajuh, J.A., Siegmund-Schultze, M., Azoulay, Y., Druyan, S. & Valle Zarate, A. 2008. Effects of the genetically reduced feather coverage in naked neck and featherless broilers on their performance under hot conditions. Poultry Science, 87, 2517-2527.

Climate data, 2021. Retrieved from https://www.climatedata.com on June 10, 2021.

Dagon, J.D. 1989. Husbandry of farm animals and poultry in the tropics. 1st edition, Rex book, Manila Philippines, 329pp.

Deka, P., Das, S., & Deka, P. 2020. Influence of maternal antibody on the efficacy of Newcastle disease vaccination in broilers. Current Journal of Applied Science and Technology, 39(7), 108-114.

Egahi, J.O., Dim, N.I., Momoh, O.M. & Gwaza D.S. 2010. Variation in qualitative traits in the Nigerian Local Chicken. International Journal of Poultry Science, 9(10), 978-979.

FAO, 2019. The future of livestock in Nigeria: Opportunities and challenges in the face of uncertainty. FAO, Rome, Italy, 46pp.

Google Maps, 2021. Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Retrieved from https://www.google.com.ng/maps/@7.223279,3.437235,14z on June 10, 2021.

Idris, M., Uddin, J., Sullivan, M., McNeill, D.M. & Phillips, C.J.C. 2021. Non-invasive physiological indicators of heat stress in cattle. Animals, 11,71.

Ikpeme, E.V., Ekerette, E.E., Efienokwu, J.N. & Ozoje, M.O. 2019. Immune response of Nigerian chicken genotypes to Salmonella and Newcastle vaccines. Trends in Applied Sciences Research, 14(4), 296-302.

Lawal, J., Jajere, S., Mustapha, M., Bello, A., Wakil, Y. & Geidam, Y. 2015. Prevalence of Newcastle disease in Gombe, Northeastern Nigeria: A ten-year retrospective study (2004–2013). British Microbiology Research Journal, 6, 367–375.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. & Mabelebele, M. 2020. Local chicken breeds of Africa: Their description, uses and conservation methods. Animals, 10, 2257.

Ogah, D.M. 2013. Canonical discriminant analysis of morphometric traits in indigenous chicken genotypes. Trakia Journal of Sciences, 11(2), 170-174.

Oke, U.K. 2011. Influence of some major genes on growth traits of local pullets in humid tropical environment. Agriculture and Biology Journal of North America, 2(4), 570-576.

Oladimeji, B.S., Osinowo, O.A., Alawa, J. & Hambolu, J.O. 1996. Estimation of average values for pulse rate, respiratory rate, rectal temperature and development of heat stress index for Yankasa sheep. *Bulletin of Health and production in Africa*, 45, 105-107.

Osei-Amponsah, R., Boa-Amponsem, K., Kayang, B.B. & Naazie, A. 2013.Characterization of primary immune response in Ghanaian local, Sasso T-44 and broiler chickens to sheep red blood cell antigens. Animal Genetic Resources, 53, 51–55.

Padhi, M.K. 2016. Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. Scientifica, 2016, 2604685.

Polgreen, P.M. & Polgreen E.L. 2018. Infectious diseases, weather and climate. Clinical infectious diseases, 66(6), 815-817.

SAS, 2002. SAS guide: Statistics released version 9.0. Statistical analysis system institute inc., Cary, NC.

Shittu, I., Joannis, T.M., Odaibo, G.N. & Olaleye, O.D. 2016. Newcastle disease in Nigeria: Epizootiology and current knowledge of circulating genotypes. VirusDisease, 27(4), 329–339.

Unigwe, C.R., Shobowale, O.M., Enibe, F., Ajayi, J.O. & Koleosho S.A. 2020. Sero-prevalence of Newcastle disease in apparently healthy normal feathered local chickens in Ido and Atiba Local Government Areas, Oyo State, Nigeria. Agro-Science, 19(4), 37-42.

Varga, P.A.T., Gonzalez, F.J.N., Landi, V., Jurado, J.M.L. & Bermejo, J.V.D. 2020. Sexual dimorphism and breed chracterisation of Creole hens through biometric canonical discriminant analysis across Ecuadorian Agroecological areas. Animals, 10, 32.

Villegas, P. & Purchase, H.G. 1989. A laboratory manual for the isolation and identification of avian pathogens.1st edition, USA Rendal Hunt, American Association of Avian pathologist, Iowa, 186–190.

Wakawa, A.M., Waziri, M.I., Aliyu, H.B., Talba, A.M., Sa'idu, L. & Abdu, P.A. 2014. Retrospective study of some viral poultry diseases diagnosed in Nigeria. International Journal of Basic and Applied Virology, 3, 16–21.

Wong, J.T., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R. & Alders, R.G. 2017. Small-scale poultry and food security in resource-poor settings: A review. Global Food Security, 15, 43-52.

Wulan, O.H., Yunita, N., Wuryastuty, H. & Wasito, R. 2017. Detection of Newcastle disease virus by immunohistochemistry on the brains of laying birds with clinical signs torticollis and curled toe paralysis. In the Veterinary Medicine International Conference, 2017, KnE life Sciences, pp 286–295.