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ORIGINAL ARTICLE

Genetic and non-genetic factors influencing KLH binding natural antibodies and specific antibody response to Newcastle disease in Kenyan chicken populations

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Abstract

This study aimed at investigating the influence of genetic and non-genetic factors on immune traits to inform on possibilities of genetic improvement of disease resistance traits in local chicken of Kenya. Immune traits such as natural and specific antibodies are considered suitable indicators of an individual's health status and consequently, used as indicator traits of disease resistance. In this study, natural antibodies binding to Keyhole Limpet Hemocyanin (KLH-NAbs) was used to measure general disease resistance. Specific antibodies binding to Newcastle disease virus (NDV-IgG) post vaccination was used to measure specific disease resistance. Titers of KLH-NAbs isotypes (KLH-IgM, KLH-IgG and KLH-IgA) and NDV-IgG were measured in 1,540 chickens of different ages ranging from 12 to 56 weeks. A general linear model was fitted to determine the effect of sex, generation, population type, phylogenetic cluster, line, genotype and age on the antibody traits. A multivariate animal mixed model was fitted to estimate heritability and genetic correlations among the antibody traits. The model constituted of non-genetic factors found to have a significant influence on the antibody traits as fixed effects, and animal and residual effects as random variables. Overall mean (±SE) concentration levels for KLH-IgM, KLH-IgG, KLH-IgA and NDV-IgG were 10.33 ± 0.04, 9.08 ± 0.02, 6.00 ± 0.02 and 10.12 ± 0.03, respectively. Sex, generation and age (linear covariate) significantly ($p < 0.05$) influenced variation across all the antibody traits. Genotype effects ($p < 0.05$) were present in all antibody traits, apart from KLH-IgA. Interaction between generation and line was significant ($p < 0.05$) in KLH-IgM and NDV-IgG while nesting phylogenetic cluster within population significantly ($p < 0.05$) influenced all antibody traits, apart from KLH-IgA. Heritability estimates for KLH-IgM, KLH-IgG, KLH-IgA and NDV-IgG were 0.28 ± 0.08, 0.14 ± 0.06, 0.07 ± 0.04 and 0.31 ± 0.06, respectively. There were positive genetic correlations (0.40–0.61) among the KLH-NAbs while negative genetic correlations (−0.26 to −0.98) were observed between the KLH-NAbs

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and NDV-IgG. Results from this study indicate that non-genetic effects due to biological and environmental factors influence natural and specific antibodies and should be accounted for to reduce bias and improve accuracy when evaluating the traits. Subsequently, the moderate heritability estimates in KLH-IgM and NDV-IgG suggest selection possibilities for genetic improvement of general and specific immunity, respectively, and consequently disease resistance. However, the negative correlations between KLH-NAbs and NDV-IgG indicate the need to consider a suitable approach that can optimally combine both traits in a multiple trait selection strategies.

KEYWORDS
chicken, genetic parameters, natural antibodies, specific antibodies

1 | INTRODUCTION

Indigenous chicken (IC) (*Gallus gallus domesticus*) plays significant roles in nutrition, food security and economic growth in many rural households in most countries in the tropical regions (Alders & Pym, 2009). In Kenya, IC account for about 80% of the total chicken population and is kept by over 75% of the rural households (Magothe et al., 2012). Their popularity, particularly among rural households, is attributed to their ability to produce under low-input systems and adapt to local environmental conditions (Olwande et al., 2010). Despite their adaptive ability, IC is predominantly raised under scavenging systems that are constrained in terms of, among other challenges, diseases that limit optimal utilization and expansion of the sub-sector (Lamont, 2010). For instance, depending on the season, disease prevalence and mortality rates, reduction in productivity and product condemnation of about 20%–100% are experienced at the farm level and these cumulatively translate to 10%–15% of annual economic losses (Okeno et al., 2011; Rist et al., 2015). With respect to climate change effects, environmental conditions are expected to favour pathogens and parasite proliferation resulting in increased disease occurrence.

Among the various poultry diseases, Newcastle disease (NCD) which is endemic among chicken in the tropics is currently considered of importance because of the massive production and economic losses it causes in the industry (Alders et al., 2018). While bio-security measures combined with vaccinations have proved useful in controlling NCD, the effects are, in some cases, temporary and/or highly influenced by the environment (Zanella, 2016). Control by use of anti-microbial drugs, on the other hand, is beneficial but often misused leading to product safety concerns (Lamont, 2010). Furthermore, the reliance on free-range scavenging system among smallholder farmers' increases transmission rates of NCD between and within flocks (Lwelamira, 2012). These factors emphasize the importance of considering alternative measures to maintain or enhance disease resistance in chicken flocks.

****Selective breeding for disease resistance, as a control measure, offers an opportunity to enhance adaptability of IC, especially, under scavenging systems where they are exposed to a myriad of disease pathogens (Cheng et al., 2013; Lwelamira et al., 2009). Besides, disease resistance is ranked a trait of economic importance among IC farmers in Kenya (Okeno et al., 2012), an indication that the trait should be considered in breeding goals. Disease resistance is generally defined as the ability to prevent infection when exposed to a pathogen or control a pathogen's life cycle (Zanella, 2016). The trait, however, is not often absolute because of the complex biological networks and host-pathogen interactions that control disease resistance, and the high sensitivity to environmental stressors (Cheng et al., 2013). On the other hand, disease resistance measurement requires that animals are challenged with pathogens and this severely violates animal welfare, presents biosecurity risks and is economically costly (Zanella, 2016). To circumvent these limitations, health traits related to the immune function are suitable indicator traits for indirect improvement of disease resistance (Cheng et al., 2013). Success of their utilization is, however, dependent on whether the traits are related to disease resistance, easy and cheap to measure and of utmost importance, heritable. Natural antibodies (NAbs) binding keyhole limpet hemocyanin (KLH-NAbs) and specific antibodies (SpAbs) binding NCD virus (NDV-IgG) have been used extensively to measure general disease resistance and specific resistance against NCD, respectively (Lwelamira, 2012; Sun et al., 2013).

Considering the intrinsic nature of innate humoral immunity, KLH-NAb titres is expected to vary among individuals (Mangino et al., 2017). Previous studies on chicken found that part of this variation was due to additive genetic effects and further estimated moderate to low (0.44–0.07) heritability for KLH-NAb isotypes (Berghof

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et al., 2015; Sun et al., 2013). Adaptive humoral immunity, on the other hand, is dependent on exogenous factors (such as vaccination) for activation, however, differences in NDV-IgG responses among individuals under controlled conditions were previously observed (Walugembe et al., 2019). This suggests possible role of genetics on NDV-IgG responses and could also be supported by the moderate (0.22–0.30) heritability estimates reported in chicken (Lwelamira et al., 2009; Touko et al., 2021). From these observations, there is evidence of heritable variation in KLH-NAbs and NDV-IgG in chicken that could be utilized for genetic improvement of general and specific disease resistance, respectively. However, the population-specific nature of heritability and its dependence on the prevailing environmental conditions indicate that these estimates may not be applicable to the chicken population of Kenya. Besides, studies on genetic components affecting antibody traits in this population are sparse.

Given that NAbs serve as a link between innate and adaptive immunity, Parmentier et al. (2004) suggests presence of a genetic basis for the functional relationship between NAbs and SpAbs. Conversely, Berghof et al. (2018) found that the magnitude and direction of genetic correlations between NAbs and SpAbs was antigen dependent. This indicates the need to determine genetic correlations between KLH-NAbs and NDV-IgG in the chicken population of Kenya prior to their inclusion in breeding objectives to prevent unfavourable correlated responses. The current study aimed at investigating the influence of genetic and non-genetic factors on KLH-NAbs and NDV-IgG, and estimate genetic correlations among the antibody traits in local chicken population of Kenya.

2 | MATERIALS AND METHODS

2.1 | Experimental population

The study was conducted at the Non-Ruminant Research Institute of the Kenya Agriculture and Livestock Research Organization (NRI-KALRO). Two populations of chicken exist at the research station; an IC population and a synthetic breed population known as KALRO chicken (KC). The IC population comprises of ecotypes from various agro-ecological zones grouped into three phylogenetic clusters based on major histocompatibility complex (MHC) linked microsatellite markers (Ngeno et al., 2015). Cluster one constitutes ecotypes from the Western and South-Rift regions that exhibit warm and humid weather; cluster two constitutes ecotypes from the North-Rift and North-Eastern regions that are considered arid and semi-arid; and cluster three constitute ecotypes from the Coastal region that is hot and humid. Within the clusters seven genetic groups exist namely normal feathered, naked neck, frizzled feathered, crested head, feathered shanks, dwarf and game-gaited (*Kuchi*) structure (Magothe et al., 2012). The synthetic (KC) population, on the other hand, originated from a dual-purpose hybrid that was subjected to true-to-type breeding (Ilatsia et al., 2017). This involved a systematic and continuous *inter-se* mating resulting to highly segregated individuals in subsequent generations. Based on plumage dominance, two distinct groups were isolated; black and white barred plumage (KC1) and black plumage (KC2). The groups were subsequently subjected to within line mating to stabilize the respective plumage colour.

The two populations are under continuous selection to develop meat (ML) and egg (EL) lines (Ilatsia et al., 2017). The ML birds are selected for body weight at 12 weeks of age (BW_{12}) (Ngeno et al., 2013). The EL are selected based on age at first egg (AFE) (Dana et al., 2011). The chicken breeding program at NRI-KALRO is in its initial stages with a small population size and limited number of pedigree and performance records, therefore, selection for ML and EL is based on phenotypic information (Ilatsia et al., 2017). The selection criterion involves retaining males and females whose phenotypic BW_{12} is at least one standard deviation above average as meat lines. Chicken that do not meet the ML criterion are considered for AFE evaluation. This involves assessing females using own phenotypic AFE records while males are assessed based on average phenotypic information on AFE from their respective daughters and dams. Individuals with below average AFE are retained. In this study, five generations of these chicken comprising of a base generation/control line (CL) and four generations of both ML and EL were considered. A total of 1540 chicken were included in the study.

Management of the experimental population followed standard operating procedures of the breeding program at NRI-KALRO (Ilatsia et al., 2017). Routine health management involved vaccination against endemic diseases namely Marek's disease (MD), NCD, and infectious bursal disease (IBD) at hatch. In addition, experimental chicken were vaccinated against fowl pox (week 6) and fowl typhoid (week 18), and dewormed and disinfected routinely.

2.2 | Data collection

Ethical approval to conduct the study was provided by the Institutional Animal Care and Use Committee (IACUC) of KALRO -Veterinary Science Research Institute (VSRI) (KALROVSRI/IACUC019/30082019). Experimental birds aged between 12 and 56 weeks were blood sampled via the wing vein. The procedure was carried out without

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anaesthesia and no chicken was killed for sample collection. Post sampling, birds were given multi-vitamins and observed for any post-trauma effects. Plasma was extracted from the blood samples for further use in antibody measurement.

Titers of natural antibody isotypes IgM, IgG and IgA binding to KLH were determined using an indirect two-step ELISA (Enzyme-linked Immunosorbent assay) as described by Berghof et al. (2015) and Khobondo et al. (2017). Specific antibody (IgG) binding to NCD virus (NDV-IgG) were measured using indirect ELISA as described by Bell and Lelenta (2002). Absorbance levels were measured at 450 nm (reference wavelength at 620 nm) using a spectrophotometer ELISA reader (mrc Scientific Instrument-UT-6100, Israel). Pre-defined serial standard dilutions of the antibody traits (Bell & Lelenta, 2002; Berghof et al., 2015) and their respective absorbance reads were used to obtain standard curves by fitting a four-parameter logistic model (Herman et al., 2008) using GraphPad Prism 9.1 (GraphPad Software). Subsequently, concentration levels of the antibody traits in plasma samples were calculated from the standard curves using their respective absorbance reads. The concentration levels of the antibody traits were thereafter adjusted to their respective sample dilution factors (1:10 for KLH-IgM, KLH-IgG, 1:40 for NDV-IgG and 1:5 for IgA) and expressed as $\log_2$ values. This was done separately for each plate to partly correct for plate differences and allow values to be comparable across plates.

2.3 | Statistical analysis

A fixed effect analysis of variance was conducted to determine non-genetic factors affecting KLH-binding NAb isotypes (IgM, IgG and IgA) and NDV-IgG. Age was fitted as a linear covariate given that chicken were sampled at different ages ranging from 16 to 56 weeks. These analyses were carried out using the *lm* and *Anova* functions from the R stats package of R Software (R Core Team, 2021). The fixed effect model was as follows:

$$\begin{aligned} Y_{ijklmno} &= \mu + S_i + G_j + L_k + P_l + C_m \\ &+ T_n + H_o + (GL)_{jk} + C(P)_{ml} + e_{ijklmno} \end{aligned} \quad (1)$$

where: $Y_{ijklmno}$ is the antibody concentration of either KLH-Nabs or NDV-IgG; μ is the overall mean; S_i is the effect of sex (i = female, male); G_j is the effect of generation (j = 1–5); L_k is the effect of line (k = control line, meat line, egg line); P_l is the effect of population (l = IC, KC); C_m is the effect of cluster group (m = C_1, C_2, C_3); T_n is the effect of genetic group (n = normal feather, naked-neck, frizzled feather, crested head, dwarf, feathered shanks, game-bird, bearded); H_o is the hatch group effect (o = 1–5); $(GL)_{jk}$ the effect of interaction between generation and line; $C(P)_{ml}$ the effect of nesting population within cluster; and $e_{ijklmno}$ is the random error term with normal distribution $N(0, \sigma^2)$, equal variance and independent distribution. Post-hoc tests of significant effects was carried out using the Tukey's test from the multcomp package of R Software (R Core Team, 2021). The adopted significance level was alpha = 0.05.

A multi-trait mixed animal model (Equation 2) was fitted to estimate genetic and phenotypic parameters of KLH-NAbs (IgM, IgG and IgA) and NDV-IgG by restricted maximum likelihood through the average information (AI-REML) algorithm using the WOMBAT software (Meyer, 2007).

$$\begin{bmatrix} \mathbf{y}_1 \\ \vdots \\ \mathbf{y}_i \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & \mathbf{X}_i \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \vdots \\ \mathbf{b}_i \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & \mathbf{Z}_i \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \vdots \\ \mathbf{a}_i \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \vdots \\ \mathbf{e}_i \end{bmatrix} \quad (2)$$

where: $\mathbf{y}_i$ is the vector of observations for the antibody traits; $\mathbf{b}_i$ the vector of fixed effects (only effects that were significant in Equation 1); $\mathbf{a}_i$ the vector of random animal additive genetic effects assumed to be $a \sim N(0, \mathbf{A}\sigma^2 a)$ in which $\mathbf{A}$ is the numerator relationship matrix and $\sigma^2 a$ is the additive genetic variance; $\mathbf{e}_i$ the vector of random residual effect assumed to be $e \sim N(0, \mathbf{I}\sigma^2 e)$ in which $\mathbf{I}$ is an identity matrix and $\sigma^2 e$ is the residual variance; $\mathbf{X}_i$, and $\mathbf{Z}_i$ are incidence matrices relating records to fixed and random animal effects, respectively. The pedigree used to construct the numerator relationship matrix consisted of 1733 individuals (inclusive of those with and without records) from five generations. Assumed covariance structures of the random model terms among the antibody traits are presented below.

$$\text{Var}\begin{bmatrix} \mathbf{a}_1 \\ \vdots \\ \mathbf{a}_i \\ \mathbf{e}_1 \\ \vdots \\ \mathbf{e}_i \end{bmatrix} = \begin{bmatrix} g_{11}\mathbf{A} \cdots g_{1i}\mathbf{A}\ 0 \ldots 0 \\ \vdots \ddots \vdots \vdots \ddots \vdots \\ g_{i1}\mathbf{A} \ldots g_{ii}\mathbf{A}\ 0 \ldots 0 \\ 0 \ldots 0\ r_{11}\mathbf{I} \ldots r_{1i}\mathbf{I} \\ \vdots \ddots \vdots \vdots \ddots \vdots \\ 0 \ldots 0\ r_{i1}\mathbf{I} \ldots r_{ii}\mathbf{I} \end{bmatrix} \quad (3)$$

where $\mathbf{a}_i$, $\mathbf{e}_i$, $\mathbf{A}$ and $\mathbf{I}$ were described in Equation 2; g_{ii} is the additive genetic variance for trait i; g_{1i} is the additive genetic covariance between trait 1 and trait i; r_{ii} is the residual variance for trait i; r_{1i} is the residual covariance between trait 1 and trait i; zero covariance between additive genetic effect and residual effect was assumed. Maternal genetic effect on the antibody traits was tested by fitting univariate animal mixed models with the effect and without the effect (Berghof et al., 2015) and thereafter, the models were compared using likelihood ratio tests. Across the traits studied, maternal genetic component had no significant ($p > 0.05$) effect and

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therefore, was not included in Equation 2. Likelihood ratio tests was used to determine whether genetic correlations among the antibody traits were significantly different from zero. On the other hand, Fisher's *r* to *z*-transformation was used to test whether phenotypic correlations were significantly different from zero with test statistic

$$z = 0.5 \ln \frac{(1+r)}{(1-r)}$$

where r is the estimated phenotypic correlation, and z follows a normal distribution with standard deviation $1/\sqrt{(n-3)}$, where n is the sample size.

3 | RESULTS

Mean concentration of the antibody traits (Table 1) showed that KLH-IgM had the highest (10.33 ng/ml) estimate while KLH-IgA had the least (6 ng/ml). Dispersion from the mean was highest in KLH-IgM (CV = 15%) while KLH-IgG had the least variation (CV = 10%). Analysis of non-genetic sources of variation (Table 1) indicated that sex and generation had significant influences on variation across all antibody traits. On the other hand, population affected KLH-IgM, KLH-IgG and NDV-IgG while effect of genetic group was significant in KLH-IgM, KLH-IgG and NDV-IgG. Hatch group effect was present on only KLH-IgA and NDV-IgG. Significant interactions between generation and line were observed in KLH-IgM and NDV-IgG while nesting of cluster within population influenced variation across the traits apart from KLH-IgA. Fitted as a linear covariate, age significantly influenced all traits.

Least square means (Table 2) of effect of sex showed that female chicken had higher estimates than male chicken in all antibody traits. Between the two chicken populations, KLH-IgM, KLH-IgG and NDV-IgG were higher ($p < 0.001$) in the IC population than in the KC population. Across generations, the mean for all antibody traits decreased from base flock through generation four. Among genetic groups, the *Kuchi* group had the highest KLH-IgM while in KLH-IgG, the *Kuchi*, naked neck and frizzled feathered genetic groups had higher ($p < 0.001$) means than the other genetic groups. In contrast, higher NDV-IgG was observed in the normal feathered, bearded, crested head and dwarf genetic groups. Cluster three within IC population

TABLE 1 Overall mean, coefficient of variation (CV) and significance levels of fixed factors that influence mean performance on KLH-binding natural antibodies and NDV-binding specific antibodies

	Antibody traits (ng/ml)[a]			
(*n* = 1540)	**KLH-IgM**	**KLH-IgG**	**KLH-IgA**	**NDV-IgG**
Overall means (±SE)[b]	10.33 ± 0.04	9.08 ± 0.02	6.00 ± 0.02	10.12 ± 0.03
CV (%)	14.71	10.02	11.33	12.84
Fixed effects	Level of significance of fixed effects on antibody traits[c]			
Sex	**	*	*	*
Population	***	**	ns	*
Cluster	ns	ns	ns	ns
Generation	***	**	**	***
Line	ns	ns	ns	ns
Genetic group	***	**	ns	**
Plate ID	***	***	ns	***
Generation * line	*	ns	ns	*
Cluster (population)	***	***	ns	**
Hatch group	ns	ns	**	***
Age[d]	***	**	***	***
Model statistics[e]	Adj. $R^2 = 0.47$ $F(22, 1139) = 48.44$ $p < 0.001$	Adj. $R^2 = 0.34$ $F(22, 1139) = 27.62$ $p < 0.001$	Adj. $R^2 = 0.12$ $F(22, 1139) = 8.33$ $p < 0.001$	Adj. $R^2 = 0.46$ $F(22, 1139) = 45.29$ $p < 0.001$

[a]ng/ml is nano-grams per millilitre concentration level of the antibody traits

[b]±SE is standard error.

[c]*ns* not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[d]Effects fitted as linear covariates.

[e]Adj. R^2 is adjusted coefficient of determination.

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TABLE 2 Least square means (±SE)[1] of factors affecting natural (KLH) and specific (NDV) antibodies

			Antibody isotypes			
Fixed effect	**Level**	***n***	**KLH-IgM**	**KLH-IgG**	**KLH-IgA**	**NDV-IgG**
Sex	Female	952	10.80 (0.13)^{a}	9.26 (0.20)^{a}	6.05 (0.16)^{a}	10.30 (0.11)^{a}
	Male	588	9.54 (0.14)^{b}	8.27 (0.21)^{b}	5.16 (0.18)^{b}	9.20 (0.12)^{b}
Population[2]	IC	1003	11.40 (0.12)^{a}	9.86 (0.24)^{a}	6.14 (0.16)^{a}	10.60 (0.11)^{a}
	KC	537	10.00 (0.23)^{b}	8.67 (0.30)^{b}	6.08 (0.18)^{a}	9.91 (0.16)^{b}
Generation	0	140	11.25 (0.27)^{a}	9.83 (0.43)^{a}	6.62 (0.34)^{a}	11.04 (0.31)^{a}
	1	287	10.40 (0.18)^{b}	9.60 (0.28)^{a}	6.29 (0.22)^{a}	10.16 (0.25)^{b}
	2	330	10.57 (0.16)^{b}	8.66 (0.23)^{b}	6.42 (0.21)^{a}	9.98 (0.18)^{b}
	3	378	8.79 (0.13)^{c}	8.75 (0.20)^{b}	5.94 (0.18)^{b}	8.87 (0.12)^{c}
	4	405	8.99 (0.10)^{c}	8.21 (0.19)^{b}	5.85 (0.17)^{b}	9.05 (0.10)^{c}
Genetic group[3]	*Kuchi*	137	11.85 (0.40)^{a}	9.91 (0.45)^{a}	6.15 (0.38)^{a}	9.46 (0.46)^{b}
	NN	206	11.24 (0.18)^{b}	9.82 (0.28)^{a}	6.00 (0.21)^{a}	9.82 (0.16)^{b}
	FR	113	11.05 (0.67)^{b}	9.65 (1.00)^{a}	5.91 (0.78)^{a}	9.67 (0.53)^{b}
	NM	429	10.40 (0.06)^{c}	8.51 (0.09)^{b}	5.89 (0.07)^{a}	11.08 (0.05)^{a}
	BD	150	10.37 (0.39)^{c}	8.68 (0.46)^{b}	6.13 (0.36)^{a}	10.79 (0.43)^{a}
	CR	319	10.26 (0.16)^{c}	8.22 (0.14)^{b}	5.75 (0.19)^{a}	10.54 (0.13)^{a}
	Dw	186	10.19 (0.35)^{c}	8.17 (0.43)^{b}	6.14 (0.31)^{a}	10.33 (0.30)^{a}
Population (Cluster)[4]						
IC	1	486	9.96 (0.13)^{c}	8.64 (0.21)^{c}	6.18 (0.12)^{a}	10.64 (0.15)^{a}
	2	309	10.62 (0.16)^{b}	9.79 (0.24)^{b}	6.02 (0.16)^{a}	10.37 (0.15)^{a}
	3	208	11.18 (0.22)^{a}	10.24 (0.26)^{a}	6.21 (0.16)^{a}	9.77 (0.17)^{b}
KC	1	282	9.05 (0.22)^{b}	8.35 (0.28)^{b}	6.00 (0.19)^{a}	10.23 (0.19)^{a}
	2	255	10.28 (0.26)^{a}	9.17 (0.29)^{a}	6.19 (0.20)^{a}	9.68 (0.22)^{b}

[1](±SE) is standard errors.

[2]IC, indigenous chicken, KC, synthetic chicken.

[3]*Kuchi* = game-gaited, BD, bearded; CR, crested head; DW, dwarf; FR, frizzle feathered; NM, normal feathered; NN, naked neck.

[4]effect of cluster nested within population. [a-c]Means (within effect) with different superscripts indicate significant differences ($p < 0.05$).

had the highest KLH-IgM and KLH-IgG followed by cluster two and cluster one. However, in NDV-IgG, cluster one and two had higher means than cluster three. Within the synthetic population, KC2 had the highest KLH-IgM and KLH-IgG while KC1 had the highest NDV-IgG.

Chicken representing the control line (generation zero) had a higher mean in KLH-IgM than those under selection for either meat or egg traits (Figure 1). In both production lines, mean estimates of KLH-IgM declined across generations with the meat line (−0.51) having a higher decay rate than the egg line (−0.23). Despite the decline, the meat line had a higher mean (11.20 ng/ml) estimate in generation one than the egg line (10.51 ng/ml). However, an interaction at generation two resulted to re-ranking of lines in subsequent generations such that the egg line had a higher mean of KLH-IgM in generations three (10.20 ng/ml) and four (10.40 ng/ml). Similarly, higher mean estimates of NDV-IgG were observed in the control line than in chicken under selection (Figure 1). In chicken under selection, there was a decline in mean estimates of NDV-IgG across generations, in which, meat lines (−0.67) had a higher a rate of decrease than the egg line (−0.40). Higher mean estimate of NDV-IgG was observed in the meat line in generation one (10.57 ng/ml) and two (10.20 ng/ml). However, an interaction at generation three showed that the egg line had a higher mean (9.50 ng/ml) estimate in generation four.

Across all traits (Table 3), residual effects (0.49–0.73) contributed more to the phenotypic variation than genetic effects (0.04–0.27). Additive genetic variability among antibody traits was highest (0.27) in NDV-IgG while KLH-IgA had the least (0.04). Residual variance was highest (0.73) in KLH-IgG and lowest (0.49) in NDV-IgG. Between the two chicken populations, additive genetic variance was higher in the IC population than the synthetic population across all antibody traits (Table 3). Higher genetic variances in KLH-IgM and NDV-IgG were observed in the control line than chicken under selection (ML and EL)

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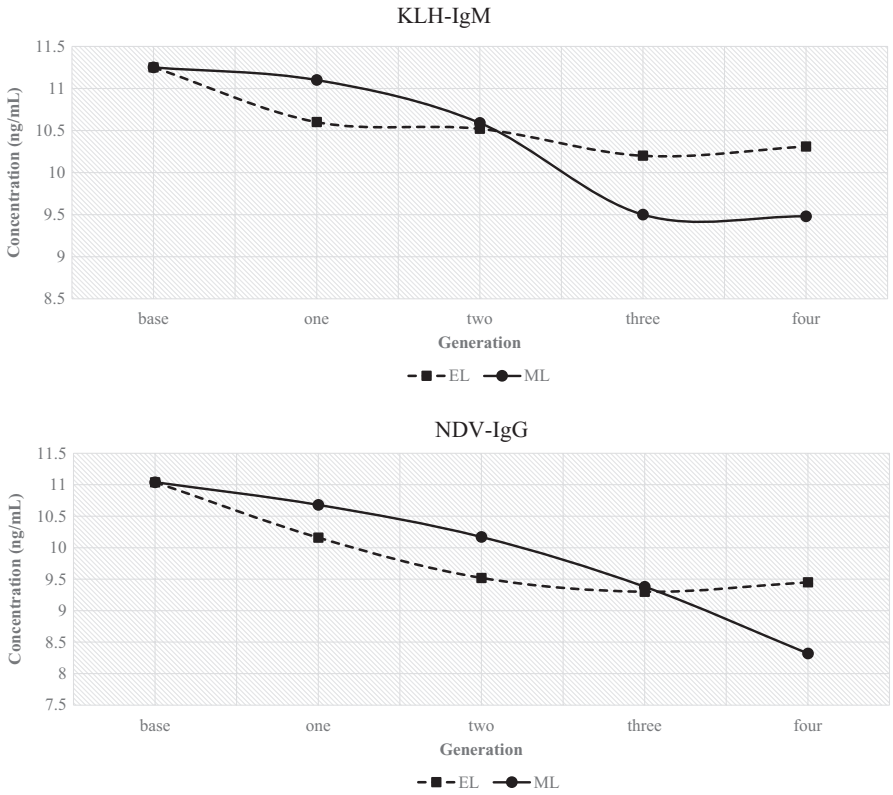

FIGURE 1 Effect of interactions between generation and line on KLH-IgM (above) and NDV-IgG (below) antibodies. (base generation = control line; EL = egg line; ML = meat line)

Genetic parameters[a]	KLH-IgM	KLH-IgG	KLH-IgA	NDV-IgG
σ^2a	0.23 (0.04)	0.10 (0.06)	0.04 (0.03)	0.27 (0.02)
σ^2e	0.51 (0.04)	0.73 (0.07)	0.59 (0.04)	0.49 (0.02)
Population[b]				
IC (n = 1003)	0.18 (0.02)	0.09 (0.04)	0.05 (0.03)	0.11 (0.01)
KC (n = 537)	0.03 (0.05)	0.02 (0.06)	0.01 (0.02)	0.02 (0.02)
Line[c]				
CL (n = 140)	0.26 (0.10)	0.11 (0.27)	0.12 (0.13)	0.33 (0.14)
ML (n = 739)	0.06 (0.04)	0.07 (0.08)	0.04 (0.06)	0.10 (0.05)
EL (n = 661)	0.18 (0.07)	0.06 (0.11)	0.08 (0.08)	0.22 (0.09)

TABLE 3 Additive genetic and residual variances (±SE) of KLH-binding natural antibodies and NDV-binding specific antibody

[a]σ^2a, additive genetic variance, σ^2e, residual variance.

[b]Additive genetic variances in the IC, indigenous chicken; KC, synthetic breed.

[c]Additive genetic variance in the CL, control line; EL, egg line; ML, meat line.

(Table 3). Between the chicken lines under selection, the egg line had a higher genetic variance in KLH-IgM and NDV-IgG than the meat line.

Across generations, estimates of genetic variance of KLH-NAbs and NDV-IgG (Figure 2) were highest (σ^2_a = 0.33–0.26) in the base population (generation zero). However, there was a steep decline at generations one (σ^2_a = 0.02–0.08) and two (σ^2_a = 0.03–0.06) in the antibody traits. Subsequently, there was an increase (σ^2_a = 0.17–0.24) in genetic variance of KLH-NAb isotypes at generation three, followed by a decrease (σ^2_a = 0.01–0.15) in the fourth generation. The decline in genetic variance of NDV-IgG continued up to generation three (σ^2_a = 0.03) and thereafter, increased to 0.28 at generation four.

Specific antibody NDV-IgG had the highest heritability (0.31) relative to KLH-NAbs (Table 4). Of the natural antibody isotypes, KLH-IgM had the highest heritability estimate (0.28) while KLH-IgA had the smallest (0.07). Genetic correlations among KLH-NAb isotypes were high and positive (0.40–0.61), however, significant (p < 0.01) associations were only observed between KLH-IgM and KLH-IgG (Table 4). On the other hand, NDV-IgG was highly negatively correlated with the KLH-IgM (−0.98) and KLH-IgG (−0.72) but was moderately correlated with

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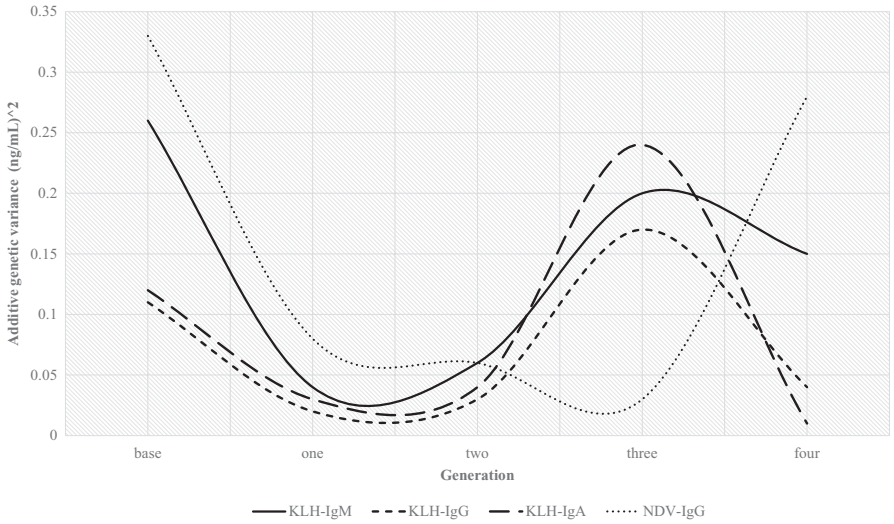

FIGURE 2 Genetic variance of KLH-natural antibodies and NDV- specific antibody across generations

TABLE 4 Estimates of heritability (diagonal), genetic (below the diagonal) and phenotypic (above the diagonal) correlations of KLH-binding natural antibodies and NDV-binding specific antibody

	KLH-IgM	KLH-IgG	KLH-IgA	NDV-IgG
KLH-IgM	**0.28 (0.08)*****	0.05 (0.03)	0.07 (0.02)*	−0.15 (0.08)
KLH-IgG	0.61 (0.22)**	**0.14 (0.06)***	0.01 (0.03)	−0.14 (0.05)
KLH-IgA	0.40 (0.15)	0.45 (0.24)	**0.07 (0.04)**	0.09 (0.03)**
NDV-IgG	−0.98 (0.21)**	−0.72 (0.32)	−0.26 (0.17)	**0.31 (0.09)*****

Note: SE in parentheses; ***Different from zero ($p < 0.001$); **Different from zero ($p < 0.01$); *Different from zero ($p < 0.05$).

KLH-IgA (−0.26). Significant ($p < 0.01$) genetic association was, however, only present between NDV-IgG and KLH-IgM. Generally, estimates of genetic correlations among the antibody traits were accompanied by high (0.17–0.32) standard errors. Phenotypic correlations among the traits followed a similar pattern as genetic correlations but with low estimates ranging from −0.15 to 0.09. However, the phenotypic correlations were only significant between KLH-IgA with KLH-IgM and NDV-IgG.

4 | DISCUSSIONS

Presence of KLH-NAbs in plasma of the study population, in absence of prior immunization with KLH antigen, confirms the poly-reactive and non-specific nature of natural antibodies (Star et al., 2007). Natural antibodies predominantly originate from CD5 B1-cells, possibly, present within peritoneal and pleural cavities and lymphoid tissues like spleen (Reyneveld et al., 2020). The B1-cells are long-lived and retain their self-renewing capacity and hence, the innate-like properties of natural antibodies. Results from this study also confirm presence of different isotypes of NAbs (IgM, IgG and IgA) and abundance of NAb-IgM among the isotypes in chicken. High level of NAb-IgM isotype is linked to its major functional contribution to innate humoral responses against foreign antigens (Vale et al., 2016). On the other hand, NAb-IgA is more involved in mucosal immunity and therefore, a higher proportion of the isotype is expected on mucosal linings (secretory NAb-IgA) than in the blood stream (systemic NAb-IgA) (Cerutti, 2008). This could explain the low levels of NAb-IgA measured in plasma in this study. Despite the low levels, presence of systemic NAb-IgA is suggested an appropriate representative of mucosal health or immunity of the birds, besides, its immunomodulatory roles (Schwartz-Albiez et al., 2009). This is because NAb-IgA produced from different sites is transported to mucosal linings of respective action sites via the blood stream (Rombout et al., 1992). Considering specific antibody IgG is the carrier of adaptive humoral immunity (Kapczynski et al., 2013), detection of NDV-IgG in the study population could imply that chicken were responsive to the on-station NCD vaccination program.

Differences in the means of antibodies between male and female birds indicate presence of sexual dimorphism in humoral immune traits. These differences could be attributed to genes on sex chromosomes (effects present throughout an individual's life) and gonadal hormones (effects apparent post sexual maturity) (Klein &

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Flanagan, 2016). For instance, Pap et al. (2010) observed that female birds had higher antibody and cell-mediated responses to immune challenges than male birds, with effects being more pronounced post-sexual maturity, an indication of hormonal effects on immune traits. Furthermore, testosterone is thought to have immunosuppressive effects due to high physiological and hormonal costs related to reproductive activity at the expense of immune functions (Furman et al., 2014). Boa-Amponsem et al. (1997), on the other hand, associated the heterogametic (ZW) nature of sex chromosomes in female chicken with higher specific antibody titers against sheep erythrocytes and higher heritability for this trait, and further suggested the immune trait may be genetically different between the sexes. However, Bovenhuis et al. (2002) estimated a genetic correlation of 0.92 between the sexes for specific antibodies and Berghof et al. (2015) found that genetic correlations between males and females for natural antibodies was not significantly different from 1. These observations indicate that, at a genetic level, natural and specific antibody traits between the two sexes are similar. Therefore, it is possible that the observed immune advantage (higher mean estimates) in female chicken over male chicken in this study may have resulted from non-additive genetic and non-genetic differences rather than additive genetic effects.

Considering that the IC and synthetic populations were subjected to the same production conditions, differences in their mean estimates for KLH-IgM, KLH-IgG and NDV-IgG could reflect genetic variability between the two populations for these traits. Higher levels of KLH-NAbs and NDV-IgG in the IC population imply higher general immune competence and specific immune response to NCD vaccination, respectively, compared to the synthetic population (Lwelamira et al., 2009; Sun et al., 2011). In a similar study, Wondmeneh et al. (2015) also suggested that high levels of KLH-NAbs in the IC population could be an adaptation mechanism to change in production conditions (free-range to confinement) when compared with an exotic layer line that is adapted to confinement. This may indicate that IC harbour a large plasticity in its immune system and hence, the ability to adapt and survive to changes and stressors in its environment (Mwacharo et al., 2013).

Within the IC population, the phylogenetic clusters used in this study (Ngeno et al., 2015) were previously associated with immune responses and disease resistance (Fulton et al., 2006). In this case, differences in antibody levels within the IC population could reflect genetic variability in MHC genomic regions among the phylogenetic clusters. In addition, differences in climatic and production conditions betweenthe on-station and the various agro-ecological zones where the phylogenetic clusters originated from may also explain the observed variation in KLH-NAb traits. For instance, higher levels of KLH-IgM and KLH-IgG in cluster three may reflect adaptive mechanisms to on-station climatic conditions. This is because the cluster comprised of chicken from the Coastal region which is hot and humid as opposed to the current study environment which is semi-arid. Furthermore, this could justify the high KLH-IgM in the *Kuchi* chicken (game-gaited stature) as it is the most dominant genetic group in cluster three originating from an isolated island with extreme climatic conditions compared to on-station conditions (Ngeno et al., 2015). Besides, the *Kuchi* chicken is known to be hardy and well adapted to extreme local conditions (Lwelamira, 2012). Major genes related to feather-reducing genetic groups have been found to not only confer adaptability to the tropical climate, but also resistance and tolerance to various diseases (Mahrous et al., 2008).This could explain the high KLH-IgM and KLH-IgG in the naked neck and frizzle feathered genetic groups. In contrast, the normal-feathered genetic group seemed to be more responsive to NCD vaccination as opposed to the other genetic groups.The presencee of variation among IC phylogenetic clusters and genetic groups for the antibody traits could be exploited to develop suitable and well-adapted lines for various environments. Within the synthetic (KC) population, variation in mean estimates of the antibody traits between KC1 and KC2 could suggest genetic divergence in immune function considering they were exposed to similar conditions. Given that the two groups were products of plumage differentiation post *inter-se* mating of an F1 hybrid flock (Ilatsia et al., 2017), genomic diversity studies would be relevant in determining the extent of variability, particularly, in the genomic regions related to the antibody traits of interest. This information might provide a concrete explanationof the observed differences in antibody traits between the two KC groups.

The differences in mean values for all the antibody traits among generations indicated a declining trend in subsequent generations. Base generation (control line) was a product of random mating while generations one to four resulted from selective breeding for production traits. It is possible that selection for production traits may have had counter effects on the immune traits given the decline in antibody levels across generations. van der Klein et al. (2015) observed negative phenotypic correlations between production and immune functions. The unfavourable associations were previously associated with trade-offs in resource distribution between the two functions (Rauw, 2012). For instance, van der Most et al. (2011) found that chicken selected for high-growth rate were associated with low-antibody responses while chicken lines selected for high-antibody responses had growth rates not

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significantly different from low-antibody response lines. The study attributed this observation to physiological differences related to resource allocation between the two traits indicating that production traits are more resource demanding than immune traits. The effect of selection for production traits on immune traits is further supported by the significant interactions between generation and line in KLH-IgM and NDV-IgG observed in this study. The linear effect of age on antibody levels suggests that the immune function improved with increase in age. Similar observations by Berghof et al. (2010) and Sun et al. (2013) indicated that exogenous stimuli from pathogens or environmental stressors an individual may encounter over time enhances the formation of antibodies. Furthermore, Bernasconi et al. (2003) also reported that these exogenous stimuli shape the antibody repertoire and levels through continuous polyclonal stimulation which initiates cross-reactivity driven responses of auto-reactive B-cell clones.

Higher additive genetic variance of the antibody traits in the IC population suggests that this population harbour large genetic plasticity for immune traits and could be exploited for selection to improve disease resistance. Besides, the IC population is previously reported to have a higher number of private alleles and heterozygosity within the MHC region (affiliated with immune responses and disease resistance) as opposed to exotic breeds (Ngeno et al., 2015; Nikbakht et al., 2013). Polymorphism in the MHC region in the IC is thought to result from natural selection imposed by adaptation to local environmental stressors (Ngeno et al., 2015). On the other hand, differences in additive genetic effect on the antibody traits between the IC and synthetic populations could be attributed to divergence in population history and genetic composition. The IC population were progenies of a non-descript founding flock from various ecotypes grouped into and mated within clusters while the synthetic population originated from an F1 dual-purpose hybrid subjected to *inter-se* mating (Ilatsia et al., 2017; Ngeno et al., 2015). However, the further genomic analysis would be necessary to ascertain the level of diversity between the two populations and its effect on immune traits.

Although divergent phenotypic selection for production traits (meat and egg lines) influenced the mean variation of KLH-IgM and NDV-IgG across generations, it is not certain that this may have had consequential effects on the additive genetic variance of the antibody traits. Nonetheless, the lower estimates of additive genetic variance in the meat and egg lines as opposed to the control line (random mating) could imply that selection for BW_{12} and AFE negatively influenced the variability of the antibody traits. Previous studies by Lwelamira et al. (2009) and van der Klein et al. (2015) found negative genetic correlations between antibody traits and growth- and egg-related traits, indicating that improved productivity would have detrimental effects on immune functions. In addition, Rauw (2012) reported that resource allocation competition among biological functions in chicken has an indirect (environmental) effect on genetic associations between production and immune-related traits. These observations suggest the need to estimate correlations between production traits under selection and antibody traits in order to determine possible genetic and environmental consequences of selection for production on immune function. On the other hand, the gradual decline in additive genetic variance across generations could infer that the diversity of genes controlling the antibody traits is reducing or that environmental effects had more influence on the antibody traits. However, the random changes in variance in generations three and four suggest otherwise. It is not certain what may have contributed to this observation, but it is possible that the founding flock of the study population may have not been in linkage disequilibrium, particularly in genomic regions associated with the antibody traits. In this case, allele frequencies of subsequent generations could be responding to any changes in the environment, such as selection or production conditions (Falconer & Mackay, 1996). With respect to selection, the study population was under phenotypic selection for production traits and it is also possible that the antibody traits may not be strongly correlated with the production traits and hence, the unstable additive genetic effects.

The low-to-moderate heritability estimates among KLH-NAbs indicate that selection for natural antibodies for genetic improvement of general immune-competence is feasible. This would also indirectly improve survivability due to the positive associations between KLH-NAbs and survival traits (Sun et al., 2011). It is presumed that production of NAb-IgM isotype is largely driven by endogenous auto-antigens acting on pre-existing B1-cells while NAb-IgG and NAb-IgA are products of external antigenic stimulation of B-cells that allows somatic hyper-mutation and isotype class switching of Nab-IgM (Xu et al., 2015). This implies that IgM are naturally present irrespective of the environmental conditions while the differentiation of IgM into IgG and IgA may reflect immune-modulating environmental influences. For instance, Berghof et al. (2010) found that immunization increased the levels of Nab-IgG and Nab-IgA as opposed to NAb-IgM. These differences in factors contributing to the production of various NAb isotypes could explain the higher additive genetic effect and heritability in KLH-IgM than in KLH-IgG and KLH-IgA. Although there was similarity in patterns of heritability across KLH-NAb isotypes, estimates from the current study were higher than those reported by Berghof et al. (2015). The variability in heritability estimates could be related to population differences and model fitted. The study by Berghof et al. (2015) used a White Leghorn population that are in general less

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diverse than the current study population. Moreover, Berghof et al. (2015) accounted for maternal environmental effects in the genetic models as opposed to the current study. In this study, it was not possible to account for maternal environmental effects due to the poor representation of dams among progenies resulting from low number of offspring per dam. On the other hand, maternal genetic effects had no significant influence on the antibody traits (results not shown) and therefore, not fitted in the multi-trait genetic model. The non-significant effect may be due to the age (12–56 weeks) of chicken at sampling which could suggest that maternal genetic effect on antibody traits is of less importance in chickens at juvenile and mature ages (Sun et al., 2013).

The moderate heritability estimate of NDV-IgG implies that the trait could be utilized for selection to improve immune responses to vaccination against NCD. Besides, Touko et al. (2015) found that chickens with high-NDV-IgG titers were associated with a higher frequency of MHC-B and non-MHC-B markers that confer resistance and tolerance to NCD. However, the heritability estimate for NDV-IgG in this study was higher than those reported in local chicken in Tanzania (Lwelamira et al., 2009) and Cameroon (Touko et al., 2021). While population variability may be a contributing factor, differences in the post-vaccination testing period could also explain the difference. In the two studies, NDV-IgG was tested two weeks post-vaccination against NCD. However, in the current study, chickens of different ages were sampled and, as such, period post-vaccination ranged from 4 to 48 weeks which was within the active period (60 weeks) of the vaccine used in the study population (van Hulten et al., 2020). Sampling period post-vaccination has an effect on antibody levels and is previously associated with different production phases of antibodies (development, maintenance and diminishing phases) which are influenced by different genetic components (Li et al., 2020; Sun et al., 2013).

Being a predictor of absolute response to selection, the magnitude of heritability estimates in this study suggest that relatively low-prediction accuracy levels are expected and as such, response to selection for the antibody traits is likely to take a longer time, especially under mass selection. This is further supported by the higher residual variances than additive genetic effects estimated across the antibody traits. Considering the studied population was raised and evaluated under controlled conditions (intensive production system), the high-residual variance may reflect residual environmental effects and/or non-additive genetic effects on the traits (Falconer & Mackay, 1996). In this situation, family selection would be a more reliable strategy as significant portion of the residual variance can be negated and hence, improve accuracies of breeding value estimations for the antibody traits (Farias et al., 2017). Besides, improved environment and management conditions (such as bio-security measures, vaccination, feeding and housing) should be considered to optimize on the effects of selective breeding, as well as, minimize disease incidences. Similarly, progenies of selected parents in the current population should perform under similar production conditions to control for possible genotype by environmental effects on the antibody traits (Rauw & Gomez-Raya, 2015). While these strategies may improve selection responses to an extent, it is known that the immune-related traits are complex and under polygenic control with each gene inducing only a small part of the overall effect (Fulton et al., 2006). For instance, according to Liu et al. (2014), many significant markers were found to influence innate and adaptive immune traits in chicken but none of them could explain more than 5% of the observed phenotypic variances. In view of these observations, Banos et al. (2020) suggest that genome-wide association studies in tropically adapted chicken would also be necessary in order to identify more genes involved in immune-related traits and possibly aid in proposing efficient selection strategies for such populations.

The positive genetic correlations among KLH-NAb isotypes imply that the traits share a common genetic background and are dependently controlled. Furthermore, the biological isotype class-switching nature of NAb-IgM to Nab-IgG and NAb-IgA could also explain the positive associations (Xu et al., 2015). Similar observations by Berghof et al. (2015) indicated that selection for a single NAb isotype is unlikely to have detrimental genetic effects on other NAb isotypes. In this case, since KLH-IgM was the most heritable NAb isotype in the current study population, selection for this trait would simultaneously improve KLH-IgG and KLH-IgA. However, significance tests of the genetic associations indicate that favourable correlated effects of selection for KLH-IgM on other isotypes would only be reliable in KLH-IgG isotype. In this regard, selection for KLH-IgM and its consequential effects on KLH-IgG is expected to improve general immune competence given NAb-IgM is central in innate humoral immunity while Nab-IgG is involved in the regulation of inflammatory reactions that aid in pathogen clearance (Panda & Ding, 2015; Schwartz-Albiez et al., 2009).

The negative genetic correlation between KLH-NAbs and NDV-IgG, on the other hand, suggest that the pleiotropic nature of genes affecting these traits is antagonistic. This implies that genetic improvement of KLH-NAbs is likely to reduce specific antibody response to vaccination against NCD. However, this would only be applicable to KLH-IgM given the significance level of genetic associations between KLH-IgM and NDV-IgG. Considering natural antibodies act as a link between innate and adaptive immunity, Parmentier et al. (2004) indicates that functional and genetic correlation between the two arms of immunity exists. However,

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Berghof et al. (2018) points out that the magnitude and direction of associations is antigen dependent. This is based on the effect of epitope/molecular pattern variation among antigens on immune-modulatory features such as pathogen recognition receptors (e.g., toll-like receptors; TLRs). TLRs are fundamental immune regulators due to their ability to recognize and bind to antigens, and activate downstream signalling T helper (Th) pathways that influence immune responses (Kawasaki & Kawai, 2014). The two antigens used in this study, KLH and NDV, vary in their molecular nature and, as such, bind to different TLRs. KLH is a glycoprotein recognized by TLR-1 which is associated with Th-2 type of immune responses while NDV is a double-stranded RNA recognized by TLR-3 which induces Th-1 type of immune responses (Berghof et al., 2018; Cheng et al., 2014). Minozzi et al. (2008) indicates that TLR-1 and TLR-3 function in opposite directions while the Th-2 pathway tends to mount stronger antibody responses than the Th-1 pathway, which is more involved in cell-mediated immune responses. In this case, it is also possible that the different TLRs and regulatory pathways between KLH-NAbs and NDV-IgG are influenced by genes that perhaps have an antagonistic association (Kimbrell & Beutler, 2001; Mangino et al., 2017). Given that KLH-NAbs and NDV-IgG play vital roles in general immunity and specific immunity against NCD, respectively, is an indication that both traits should be considered in breeding objectives intended to improve disease resistance in chicken. The antagonistic genetic correlations, however, suggest that multiple trait selection using optimal weights and optimal breeding strategies need to be considered in order to simultaneously improve KLH-NAbs and NDV-IgG.

The rather high standard errors of genetic correlation estimates are likely to have resulted from the relatively small sample size used in this study. In this case, the estimates may not have sufficient reliability and hence, these results should be applied with caution while further analysis with a larger sample size is necessary to improve accuracy of genetic correlation estimates. Generally, lower phenotypic correlations than genetic correlations among the antibody traits could suggest that these traits do not share common environmental effects or have different responsiveness to environmental influences. Furthermore, although not presented in the results, residual correlations among the antibody traits were not significantly different from zero and could also indicate that the traits are under independent control of residual environmental effect or non-additive genetic effect.

5 | CONCLUSIONS

Non-genetic factors related to sex, population, phylogenetic cluster, generation, line, genotype and age significantly influenced KLH-NAbs and NDV-IgG, and therefore, should be accounted for to reduce bias and improve accuracy when evaluating natural and specific antibodies in chicken. Subsequently, the considerable amount of additive genetic variation in KLH-IgM and NDV-IgG traits implies selection possibilities for humoral immune traits. However, the low-to-moderate heritability estimates indicate that relatively low-accuracy levels would be expected and hence, reduced genetic gains. Genetic correlations among the KLH-NAb isotypes were favourable, which suggests that NAb isotypes can be improved simultaneously. In contrast, KLH-NAbs were negatively correlated with NDV-IgG implying that genetic improvement of natural antibodies would be associated with low-specific antibodies binding NDV. Despite the antagonistic correlations, multiple trait selection using optimal weights and optimal breeding strategies need to be considered in order to genetically improve KLH-NAbs and NDV-IgG simultaneously. The high standard errors of genetic correlation estimates, however, indicate the need for further analysis with a larger sample size prior to inclusion of these traits in breeding objectives. Results from this study provide a better understanding of factors affecting natural and specific antibody traits in a heterogeneous chicken population, and may enable effective decisions prior to inclusion of immune parameters in breeding programs intended for tropically adapted chicken.

AUTHORS' CONTRIBUTION

Sophie Miyumo: Conceptualization (lead); methodology (lead); investigation (lead); formal analysis (lead); writing – original draft (lead); visualization (lead); writing – review and editing (equal). **Chrilukovian B. Wasike:** Methodology (supporting); writing – original draft (supporting); writing – review and editing (equal). **Evans D. Ilatsia:** Conceptualization (supporting); funding acquisition (lead); writing – review and editing (equal). **Jörn Bennewitz:** writing – review and editing (equal); supervision (lead). **Mizeck G. Chagunda:** writing – review and editing (equal); supervision (lead).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Kenya Agricultural and Livestock Research Organization. Restrictions apply to the availability of these data, which were used under license for this study. Data are available on request from the corresponding author with the permission of the Kenya Agricultural and Livestock Research Organization.

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REFERENCES

Alders, R. G., Dumas, S. E., Rukambile, E., Magoke, G., Maulaga, W., Jong, J., & Costa, R. (2018). Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. *Maternal & Child Nutrition*, *14*, e12668. https://doi.org/10.1111/mcn.12668

Alders, R. G., & Pym, R. A. E. (2009). Village poultry: Still important to millions, eight thousand years after domestication. *World's Poultry Science Journal*, *65*(2), 181–190. https://doi.org/10.1017/S0043933909000117

Banos, G., Lindsay, V., Desta, T. T., Bettridge, J., Sanchez-Molano, E., Vallejo-Trujillo, A., Matika, O., Dessie, T., Wigley, P., Christley, R. M., Kaiser, P., Hanotte, O., & Psifidi, A. (2020). Integrating genetic and genomic analyses of combined health data across ecotypes to improve disease resistance in indigenous African chickens. *Frontiers in Genetics*, *11*, 1180. https://doi.org/10.3389/FGENE.2020.543890

Bell, J. G., & Lelenta, M. (2002). An ELISA for the detection of antibodies against Newcastle disease virus in African village poultry. *International Atomic Energy Agency (IAEA)*, 174–178.

Berghof, T. V. L., De Vries Reilingh, G., Nieuwland, M. G. B., & Parmentier, H. K. (2010). Effect of aging and repeated intratracheal challenge on levels of cryptic and overt natural antibodies in poultry. *Poultry Science*, *89*(2), 227–235. https://doi.org/10.3382/ps.2009-00449

Berghof, T. V. L., van der Klein, S. A. S., Arts, J. A. J., Parmentier, H. K., van der Poel, J. J., & Bovenhuis, H. (2015). Genetic and non-genetic inheritance of natural antibodies binding keyhole limpet hemocyanin in a purebred layer chicken line. *PLoS One*, *10*(6), 1–13. https://doi.org/10.1371/journal.pone.0131088

Berghof, T. V. L., Visker, H. P. W., Arts, J. A. J., Parmentier, H. K., van der Poel, J. J., Vereijken, A. L. J., & Bovenhuis, H. (2018). Genomic region containing toll-like receptor genes has a major impact on total IGM antibodies including KLH-binding IGM natural antibodies in chickens. *Frontiers in Immunology*, *8*, 1879. https://doi.org/10.3389/FIMMU.2017.01879

Bernasconi, N. L., Onai, N., & Lanzavecchia, A. (2003). A role for toll-like receptors in acquired immunity: Up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood*, *101*(11), 4500–4504. https://doi.org/10.1182/BLOOD-2002-11-3569

Boa-Amponsem, K., Dunnington, E. A., & Siegel, P. B. (1997). Genetic architecture of antibody responses of chickens to sheep red blood cells. *Journal of Animal Breeding and Genetics*, *114*(1–6), 443–449. https://doi.org/10.1111/J.1439-0388.1997.TB00530.X

Bovenhuis, H., Bralten, H., Nieuwland, M. G. B., & Parmentier, H. K. (2002). Genetic parameters for antibody response of chickens to sheep red blood cells based on a selection experiment. *Poultry Science*, *81*, 309–315. https://doi.org/10.1093/ps/81.3.309

Cerutti, A. (2008). The regulation of IgA class switching. *Nature Reviews Immunology*, *8*(6), 421–434. https://doi.org/10.1038/nri2322

Cheng, H. H., Kaiser, P., & Lamont, S. J. (2013). Integrated genomic approaches to enhance genetic resistance in chickens. *Annual Review of Animal Biosciences*, *1*, 239–260. https://doi.org/10.1146/ANNUREV-ANIMAL-031412-103701

Cheng, J., Sun, Y., Zhang, X., Zhang, F., Zhang, S., Yu, S., Qiu, X., Tan, L., Song, C., Gao, S., Wu, Y., & Ding, C. (2014). Toll-like receptor 3 inhibits Newcastle disease virus replication through activation of pro-inflammatory cytokines and the type-1 interferon pathway. *Archives of Virology*, *159*(11), 2937–2948. https://doi.org/10.1007/S00705-014-2148-6

Dana, N., van der Waaij, E. H., & van Arendonk, J. A. M. (2011). Genetic and phenotypic parameter estimates for body weights and egg production in Horro chicken of Ethiopia. *Tropical Animal Health and Production*, *43*(1), 21–28. https://doi.org/10.1007/S11250-010-9649-4

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*. Longman.

Farias, T. F., Cesar, J. R. D. O., & Silva, L. P. D. (2017). Methods of selection using the quantitative genetics in aquaculture-A short review. *Insights in Aquaculture and Biotechnology*, *1*(1), 1–8. https://www.imedpub.com/articles/methods-of-selection-using-the-quantitative-genetics-in-aquaculturea-short-review.pdf

Fulton, J. E., Juul-Madsen, H. R., Ashwell, C. M., McCarron, A. M., Arthur, J. A., O'Sullivan, N. P., & Taylor, R. L. (2006). Molecular genotype identification of the Gallus gallus major histocompatibility complex. *Immunogenetics*, *58*(5–6), 407–421. https://doi.org/10.1007/S00251-006-0119-0

Furman, D., Hejblum, B. P., Simon, N., Jojic, V., Dekker, C. L., Thiébaut, R., Tibshirani, R. J., & Davis, M. M. (2014). Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proceedings of the National Academy of Sciences*, *111*(2), 869–874. https://doi.org/10.1073/pnas.1321060111

Herman, R. A., Scherer, P. N., & Shan, G. (2008). Evaluation of logistic and polynomial models for fitting sandwich-ELISA calibration curves. *Journal of Immunological Methods*, *339*(2), 245–258. https://doi.org/10.1016/J.JIM.2008.09.001

Ilatsia, E. D., Magothe, T. M., Alaru, P. A. O., Okeno, T. O., Muasya, T. K., Ngeno, K., Miyumo, S., & Muhuyi, W. B. (2017). A breeding programme for improvement and conservation of the Kenyan indigenous chicken. *The ASAL-APRP End of Project Conference and Exhibition, 25–27 April 2017*, KALRO Headquarters, Nairobi-Kenya.

Kapczynski, D. R., Afonso, C. L., & Miller, P. J. (2013). Immune responses of poultry to Newcastle disease virus. *Developmental & Comparative Immunology*, *41*(3), 447–453. https://doi.org/10.1016/J.DCI.2013.04.012

Kawasaki, T., & Kawai, T. (2014). Toll-like receptor signaling pathways. *Frontiers in Immunology*, *5*, 461. https://doi.org/10.3389/FIMMU.2014.00461

14390388, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbg.12738 by EBMG ACCESS - KENYA, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Khobondo, J. O., Mwakubambanya, R., Wasike, C. B., & Kahi, A. K. (2017). Genetic and non-genetic sources of variation in natural antibodies titre values among indigenous chicken. *American Journal of Research Communication*, *5*(7), 31–45.

Kimbrell, D. A., & Beutler, B. (2001). The evolution and genetics of innate immunity. *Nature Reviews Genetics*, *2*(4), 256–267.

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626–638. https://doi.org/10.1038/nri.2016.90

Lamont, S. J. (2010). Genetics of disease resistance. *Adapting animal production to changes for a growing human population*, 83.

Li, R. F., Liu, S. P., Yuan, Z. H., Yi, J. E., Tian, Y. N., Wu, J., & Wen, L. X. (2020). Effects of induced stress from the live LaSota Newcastle disease vaccination on the growth performance and immune function in broiler chickens. *Poultry Science*, *99*(4), 1896–1905. https://doi.org/10.1016/j.psj.2019.12.004

Liu, T., Qu, H., Luo, C., Li, X., Shu, D., Lund, M. S., & Su, G. (2014). Genomic selection for the improvement of antibody response to Newcastle disease and avian influenza virus in chickens. *PLoS One*, *9*(11), 1–6. https://doi.org/10.1371/journal.pone.0112685

Lwelamira, J. (2012). Phenotypic and genetic parameters for body weights and antibody response against Newcastle disease virus (NDV) vaccine for Kuchi chicken ecotype of Tanzania under extensive management. *Tropical Animal Health and Production*, *44*(7), 1529–1534. https://doi.org/10.1007/s11250-012-0099-z

Lwelamira, J., Kifaro, G. C., & Gwakisa, P. S. (2009). Genetic parameters for body weights, egg traits and antibody response against Newcastle disease virus (NDV) vaccine among two Tanzania chicken ecotypes. *Tropical Animal Health and Production*, *41*(1), 51–59. https://doi.org/10.1007/s11250-008-9153-2

Magothe, T. M., Okeno, T. O., Muhuyi, W. B., & Kahi, A. K. (2012). Indigenous chicken production in Kenya: Current status. *World's Poultry Science Journal*, *68*(1), 119–132. https://doi.org/10.1017/s0043933912000128

Mahrous, M., Fathi, M. M., Galal, A. A., & El-Dein, A. Z. (2008). Impact of naked neck (Na) and frizzle (F) genes on growth performance and immunocompetence in chickens Article in. *International Journal of Poultry Science*, *7*(1), 45–54. https://doi.org/10.3923/ijps.2008.45.54

Mangino, M., Roederer, M., Beddall, M. H., Nestle, F. O., & Spector, T. D. (2017). ARTICLE innate and adaptive immune traits are differentially affected by genetic and environmental factors. *Nature Communications*, *8*, 1–7. https://doi.org/10.1038/ncomms13850

Meyer, K. (2007). WOMBAT—A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). *Journal of Zhejiang University Science B*, *8*(11), 815–821. https://doi.org/10.1631/JZUS.2007.B0815

Minozzi, G., Parmentier, H. K., Mignon-Grasteau, S., Nieuwland, M. G. B., Bed'hom, B., Gourichon, D., Minvielle, F., & Pinard-van der Laan, M. H. (2008). Correlated effects of selection for immunity in white Leghorn chicken lines on natural antibodies and specific antibody responses to KLH and M. butyricum. *BMC Genetics*, *9*, 1–13. https://doi.org/10.1186/1471-2156-9-5

Mwacharo, J. M., Nomura, K., Hanada, H., Han, J. L., Amano, T., & Hanotte, O. (2013). Reconstructing the origin and dispersal patterns of village chickens across East Africa. *Molecular Ecology*, *22*(10), 2683–2697. https://doi.org/10.1111/mec.12294

Ngeno, K., Magothe, T. M., Okeno, T. O., Bebe, B. O., & Kahi, A. K. (2013). Heritabilities and correlations between body weights and growth curve parameters of indigenous chicken populations reared intensively in Kenya. *Research Journal of Poultry Sciences*, *6*(3), 43–52.

Ngeno, K., Waaij, E. H van der., Megens, H. J., Kahi, A. K., Arendonk, J. A. M. van, & Crooijmans, R. P. M. A. (2015). Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers. *Animal Genetic Resources*, *56*, 1–7. https://doi.org/10.1017/S2078633614000484.

Nikbakht, G., Esmailnejad, A., & Barjesteh, N. (2013). LEI0258 microsatellite variability in Khorasan, Marandi, and Arian chickens. *Biochemical Genetics*, *51*(5–6), 341–349. https://doi.org/10.1007/S10528-013-9567-Z

Okeno, T. O., Bett, R. C., & Peters, K. J. (2011). Economic values for resistance to Helminthosis and Newcastle disease in indigenous chicken in the tropics. *Journal of Veterinary Advances*, *1*(1), 1–10.

Okeno, T. O., Kahi, A. K., & Peters, K. J. (2012). Characterization of indigenous chicken production systems in Kenya. *Tropical Animal Health and Production*, *44*(3), 601–608. https://doi.org/10.1007/S11250-011-9942-X

Olwande, P. O., Ogara, W. O., Okuthe, S. O., Muchemi, G., Okoth, E., Odindo, M. O., & Adhiambo, R. F. (2010). Assessing the productivity of indigenous chickens in an extensive management system in southern Nyanza, Kenya. *Tropical Animal Health and Production*, *42*(2), 283–288. https://doi.org/10.1007/S11250-009-9418-4

Panda, S., & Ding, J. (2015). Natural antibodies bridge innate and adaptive immunity. *Journal of Immunology*, *194*, 13–20. https://doi.org/10.4049/jimmunol.1400844

Pap, P. L., Czirják, G. Á., Vágási, C. I., Barta, Z., & Hasselquist, D. (2010). Sexual dimorphism in immune function changes during the annual cycle in house sparrows. *Naturwissenschaften*, *97*(10), 891–901. https://doi.org/10.1007/S00114-010-0706-7

Parmentier, H. K., Lammers, A., Hoekman, J. J., Reilingh, G. D. V., Zaanen, I. T. A., & Savelkoul, H. F. J. (2004). Different levels of natural antibodies in chickens divergently selected for specific antibody responses. *Developmental & Comparative Immunology*, *28*(1), 39–49. https://doi.org/10.1016/S0145-305X(03)00087-9

R Core Team. (2021) R: Language and environment for statistical computing. https://www.r-project.org/.

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, *3*(DEC), 267. https://doi.org/10.3389/FGENE.2012.00267/FULL

Rauw, W. M., & Gomez-Raya, L. (2015). Genotype by environment interaction and breeding for robustness in livestock. *Frontiers in Genetics*, *6*, 310. https://doi.org/10.3389/FGENE.2015.00310

Reyneveld, G. I., Savelkoul, H. F. J., & Parmentier, H. K. (2020). Current understanding of natural antibodies and exploring the possibilities of modulation using veterinary models, A Review. *Frontiers in Immunology*, *11*, 2139. https://doi.org/10.3389/FIMMU.2020.02139

Rist, C., Ngonghala, C., & Garchitorena, A. (2015). Modeling the burden of poultry disease on the rural poor in Madagascar. *One Health*, *1*, 60–65. https://www.sciencedirect.com/science/article/pii/S2352771415000117

Rombout, J. H. W. M., Sijtsma, S. R., West, C. E., Karabinis, Y., Sijtsma, O. K. W., Zijpp, A. J., & Koch, G. (1992). Effect of vitamin A deficiency and Newcastle disease virus infection on

14390388, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jbg.12738 by EBMG ACCESS - KENYA, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

IgA and IgM secretion in chickens. *British Journal of Nutrition*, *68*(3), 753–763. https://doi.org/10.1079/BJN19920131

Schwartz-Albiez, R., Monteiro, R. C., Rodriguez, M., Binder, C. J., & Shoenfeld, Y. (2009). Natural antibodies, intravenous immunoglobulin and their role in autoimmunity, cancer and inflammation. *Clinical and Experimental Immunology*, *158*, 43–50. https://doi.org/10.1111/J.1365-2249.2009.04026.X

Star, L., Frankena, K., Kemp, B., Nieuwland, M. G. B., & Parmentier, H. K. (2007). Natural humoral immune competence and survival in layers. *Poultry Science*, *86*(6), 1090–1099. https://doi.org/10.1093/ps/86.6.1090

Sun, Y., Biscarini, F., Bovenhuis, H., Parmentier, H. K., & Van Der Poel, J. J. (2013). Genetic parameters and across-line SNP associations differ for natural antibody isotypes IgM and IgG in laying hens. *Animal Genetics*, *44*(4), 413–424. https://doi.org/10.1111/AGE.12014

Sun, Y., Parmentier, H. K., Frankena, K., & van der Poel, J. J. (2011). Natural antibody isotypes as predictors of survival in laying hens. *Poultry Science*, *90*(10), 2263–2274. https://doi.org/10.3382/ps.2011-01613

Touko, B. A., Keambou, C. T., Han, J. M., Bembide, C., Skilton, R. A., Ogugo, M., Manjeli, Y., Osama, S., Cho, C. Y., & Djikeng, A. (2015). Molecular typing of the major histocompatibility complex B microsatellite haplotypes in Cameroon chicken. *Animal Genetic Resources*, *56*, 47–54. https://doi.org/10.1017/S2078633614000538

Touko, B. A. H., Mbiydzenyuy, A. T. K., Tumasang, T. T., & Awah-Ndukum, J. (2021). Heritability estimate for antibody response to vaccination and survival to a Newcastle disease infection of native chicken in a low-input production system. *Frontiers in Genetics*, *12*, 6666947. https://doi.org/10.3389/fgene.2021.666947

Vale, A. M., Cavazzoni, C. B., Nobrega, A., & Schroeder, H. W. (2016). The global self-reactivity profile of the natural antibody repertoire is largely independent of germline DH sequence. *Frontiers in Immunology*, *7*, 296. https://doi.org/10.3389/FIMMU.2016.00296

van der Klein, S. A. S., Berghof, T. V. L., Arts, J. A. J., Parmentier, H. K., Van der Poel, J. J., & Bovenhuis, H. (2015). Genetic relations between natural antibodies binding keyhole limpet hemocyanin and production traits in a purebred layer chicken line. *Poultry Science*, *94*(5), 875–882. https://doi.org/10.3382/ps/pev052

van der Most, P. J., De Jong, B., Parmentier, H. K., & Verhulst, S. (2011). Trade-off between growth and immune function: A meta-analysis of selection experiments. *Functional Ecology*, *25*(1), 74–80. https://doi.org/10.1111/j.1365-2435.2010.01800.x

van Hulten, M. C. W., Cruz-Coy, J., Gergen, L., Pouwels, H., ten Dam, G. B., Verstegen, I., de Groof, A., Morsey, M., & Tarpey, I. (2020). Efficacy of a Turkey herpesvirus double construct vaccine (HVT-ND-IBD) against challenge with different strains of Newcastle disease, infectious bursal disease and Marek's disease viruses. *Avian Pathology*, *50*(1), 18–30. https://doi.org/10.1080/03079457.2020.1828567

Walugembe, M., Mushi, J. R., Amuzu-Aweh, E. N., Chiwanga, G. H., Msoffe, P. L., Wang, Y., Saelao, P., Kelly, T., Gallardo, R. A., Zhou, H., Lamont, S. J., Muhairwa, A. P., & Dekkers, J. C. M. (2019). Genetic analyses of Tanzanian local chicken ecotypes challenged with Newcastle disease virus. *Genes*, *10*(7), 546. https://doi.org/10.3390/GENES10070546

Wondmeneh, E., Van Arendonk, J. A. M., Van Der Waaij, E. H., Ducro, B. J., & Parmentier, H. K. (2015). High natural antibody titers of indigenous chickens are related with increased hazard in confinement. *Poultry Science*, *94*(7), 1493–1498. https://doi.org/10.3382/ps/pev107

Xu, X., Ng, S. M., Hassouna, E., Warrington, A., Oh, S. H., & Rodriguez, M. (2015). Human-derived natural antibodies: Biomarkers and potential therapeutics. *Future Neurology*, *10*(1), 25–39.

Zanella, R. (2016). Genomic tools and animal health. *Veterinary Sciences*, *3*(3), 21. https://doi.org/10.3390/vetsci3030021

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