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### Cover Page Footnote

The authors appreciate the Redeemer's University Africa Center of Excellence for Genomics of Infectious Diseases (ACEGID) for providing a facility in which to conduct this research. We would like to acknowledge the advisory support that was provided by Dr. Ogunkanmi, B.O. of the Department of Cell Biology and Genetics, University of Lagos.

## Sequence Variability in the *NRAMP1* Gene among Indigenous and Exotic Chicken Types

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### Abstract

The natural resistance-associated macrophage protein 1 (*NRAMP1*) gene in chickens, which exists on chromosome 7, is thought to play a significant role in disease resistance. Variations in this gene have been documented and have played crucial roles in the variations in the resistance and/or susceptibility that is expressed by individuals and different groups of animal species. In this study, the *NRAMP1* gene was studied for single nucleotide polymorphism (SNP) variability between indigenous and exotic chicken breeds. The gene was amplified using the polymerase chain reaction (PCR) method and sequenced and analyzed. Six SNPs, both synonymous and non-synonymous (C3700T, G3702C, A3712C, C3714G, C3693G, and G3705T), were detected in the gene in both indigenous and exotic chicken types. One allele form was detected among all the sample animals that were studied. A phylogenetic tree revealed that the indigenous chicken type and the exotic broiler chicken type are genetically similar with respect to this gene. However, the exotic layer chicken type is genetically suggested to be distantly related to both the indigenous and broiler chicken types, indicating that the gene has probably been evolving both within and among different poultry species.

*Keywords: chicken, NRAMP1 gene, nucleotide variation, phylogenic tree*

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### Introduction

An important factor that negatively influences animal husbandry is disease. Resistance to different diseases varies among poultry breeds and types. This is highly determined by either the genotype or the presence of a specific allele type [1]. Several studies have suggested the strong influence of genetic factors in the control of resistance and susceptibility of poultry to infections. The natural resistance-associated macrophage protein 1 (*NRAMP1*) gene's role has been significantly implicated among many poultry types [1–5].

The *NRAMP1* gene has been identified as a functional candidate gene that has disease resistance activity in a number of animal species [3] and in human populations [6, 7]. It has also been implicated in the control of host resistance to pathogenic infections in some animals such as chicken (*Gallus* spp), guinea fowl (*Numida meleagris*) and turkey (*Meleagris gallopavo*) [4, 8]. *NRAMP1* is produced in intracellular vesicular membranes; in the presence of pathogens, it is transferred to the pathogen's membrane. The gene functions by removing iron ions from macrophages and thus influences the growth of intracellular pathogens [9,

10]. In chicken, the *NRAMP1* gene exists on chromosome 7, comprises about 5kb of the genomic DNA, and contains 15 exons. The chicken *NRAMP1* polypeptide is said to encode a 555-amino-acid residue membrane protein with 12 putative transmembrane domains, two *N*-linked glycosylation sites, and an evolutionary conserved consensus transport motif [2, 11]. Among chicken, mouse, and human *NRAMP1* genes, the peptide sequence similarity is 68% [2]. This gene also contains one major and two minor transcription initiation sites, a classical TATAA element and consensus sequences for binding the myeloid specific PU1 factor, several lipopolysaccharides (NF-IL6 and NF-κB), and interferon-γ-inducible response elements, which are also contained in the gene's promoter region [2, 11]. Polymorphisms in the gene in poultry have been associated with different exhibited immune traits [1]. Different genotypes of this gene influence the microglia cell-mediated anti-microbial function. A genotype may show resistance, and another genotypic form may be susceptible to certain infections [8, 12]. Girard-Santosuosso *et al.* [13] showed that different chicken populations display different heritability of susceptibility to infection due to the existence of genetic polymorphism in the *NRAMP1* gene.

The comparison of indigenous chicken types with exotic types reveals a reservoir of diverse economically important traits, including hardiness and resistance to different avian pathogenic infections, and genes [14]. According to the Food and Agriculture Organization of the United Nations (FAO), global poultry conservation programs, especially those for indigenous breeds, are few, and the FAO has shown concern over the total replacement of indigenous breeds with exotics [15, 16]. Therefore, the conservation of poultry's innate immunity to disease is an essential tool for future development of the poultry industry [17, 18]. Hence, in this study, the *NRAMP1* gene was considered to explore variations in the gene between indigenous and exotic poultry types.

## Material and Methodology

**Study population.** A total of 257 indigenous (reared to reproduce, for flesh (meat), and for egg production) and exotic (Broiler: reared for flesh (meat) production and Layer (Isa brown: reared primarily for egg production)) chicken types of reproductive age were used for this study.

Whole blood was collected from the wing vein of each bird using a 1-mL syringe. The blood was then transferred into a systematically labeled ethylenediamine tetraacetic acid (EDTA) tube that contained anticoagulants.

**Genomic DNA extraction.** Genomic DNA extraction was done by following a modified protocol of Sambrook *et al.*'s work [19]. The procedure was as follows:

Genomic DNA were extracted from 50  $\mu$ l of blood. In a 1.5-ml tube, 700  $\mu$ l of lysis buffer (10mM Tris -HCl pH = 8.0, 100mM NaCl, 1 mM EDTA, pH = 8.0, 0.5 % Sodium Dodecyl Sulphate) were added to the blood with a gentle shake and incubated at 58–60 °C for 60–70 minutes. A volume of 330 $\mu$ l of phenol-chloroform-isoamylalcohol (P:C:I = 25:24:1) mix was added and centrifuged at 12,000 rpm for 5 minutes at 4 °C. The supernatant was collected in a new, labelled tube, and 300–330  $\mu$ l of P:C:I mix (P:C:I/supernatant) were added for the second wash and centrifuged at 12,000 rpm for 5 minutes at 4 °C. The supernatant was transferred into a new, labelled tube, and 360 $\mu$ l of ice-cold isopropanol were added to allow the DNA to precipitate (if no precipitation occurred, the set was left at -20 °C overnight). The solution was centrifuged at 10,000 rpm for 5 minutes at 4 °C, the liquid phase was decanted, and the DNA was washed with ice-cold 70% alcohol and spun for 5 minutes at 10,000rpm (4 °C). The liquid was drained and dried at room temperature, and the DNA was dissolved in 40  $\mu$ l of 1x low salt Tris EDTA (TE).

**Gene amplifications (PCR).** A polymerase chain reaction (PCR) was performed in a total volume of 25

$\mu$ l, containing 5  $\mu$ l of DNA, 1  $\mu$ l of each primer (10  $\mu$ M), and 12.5  $\mu$ l of the PCR master mix (One Taq Quick-load 2x master mix with standard buffer, New England BioLab, USA). Amplification of the *NRAMP1* fragment was done using the primer: forward 5'GGCGTCATCCTGGGCTGCTAT3' and reverse 5'AGACCGTTGGCGAAGTCATGC3'. The PCR conditions were 95 °C for 5 min before the first cycle, then 94 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 40 sec for 35 cycles, followed by 72 °C for 10 min at 4 °C.

**Gel electrophoresis.** The PCR amplicons were analyzed by electrophoresis with 2% agarose gel at 90 volts for 45 minutes, after which the band patterns were observed under UV light.

**Nucleotide sequencing.** The PCR products were sequenced using the ABI 3500XL Genetic Analyzer (Inqaba Biotech South Africa).

**Nucleotide sequence reads analysis.** The obtained sequences were first checked manually and verified by using BLAST to ascertain that they were of the *NRAMP1* gene. The related sequences from the identified reference species were retrieved from Genbank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Corresponding regions were cut and saved. Subsequently, all the sequences were aligned by CLUSTALW, and variations among the nucleotide sequences were estimated using Molecular Evolutionary Genetic Analysis (MEGA Version 7.0).

The genetic distance between bird types based on nucleotide sequence variability in the gene were estimated as the Kimura 2-parameter distance using MEGA software. Phylogenetic trees were constructed using cumulative nucleotide sequence variability in the genes with the neighbor joining method (MEGA Version 7.0). Support of the clusters was evaluated by bootstrap as a percentage recurrence of clusters based on 1,000 bootstrapped replications with MEGA Version 7.0.

## Results

The fragment size of the *NRAMP1* gene, when amplified, was 900bp. Among all the chicken breeds, the *NRAMP1* gene was found to be non-polymorphic, and only one allele form was detected.

A sequence analysis of the *NRAMP1* gene among the studied chicken types revealed six single nucleotide polymorphisms (SNPs) at various positions: C3700T and G3702C, with an amino acid change from leucine to phenylalanine, A3712C and C3714G, with the same amino acid change from isoleucine to leucine, and C3693G and G3705T, with no amino acid change.

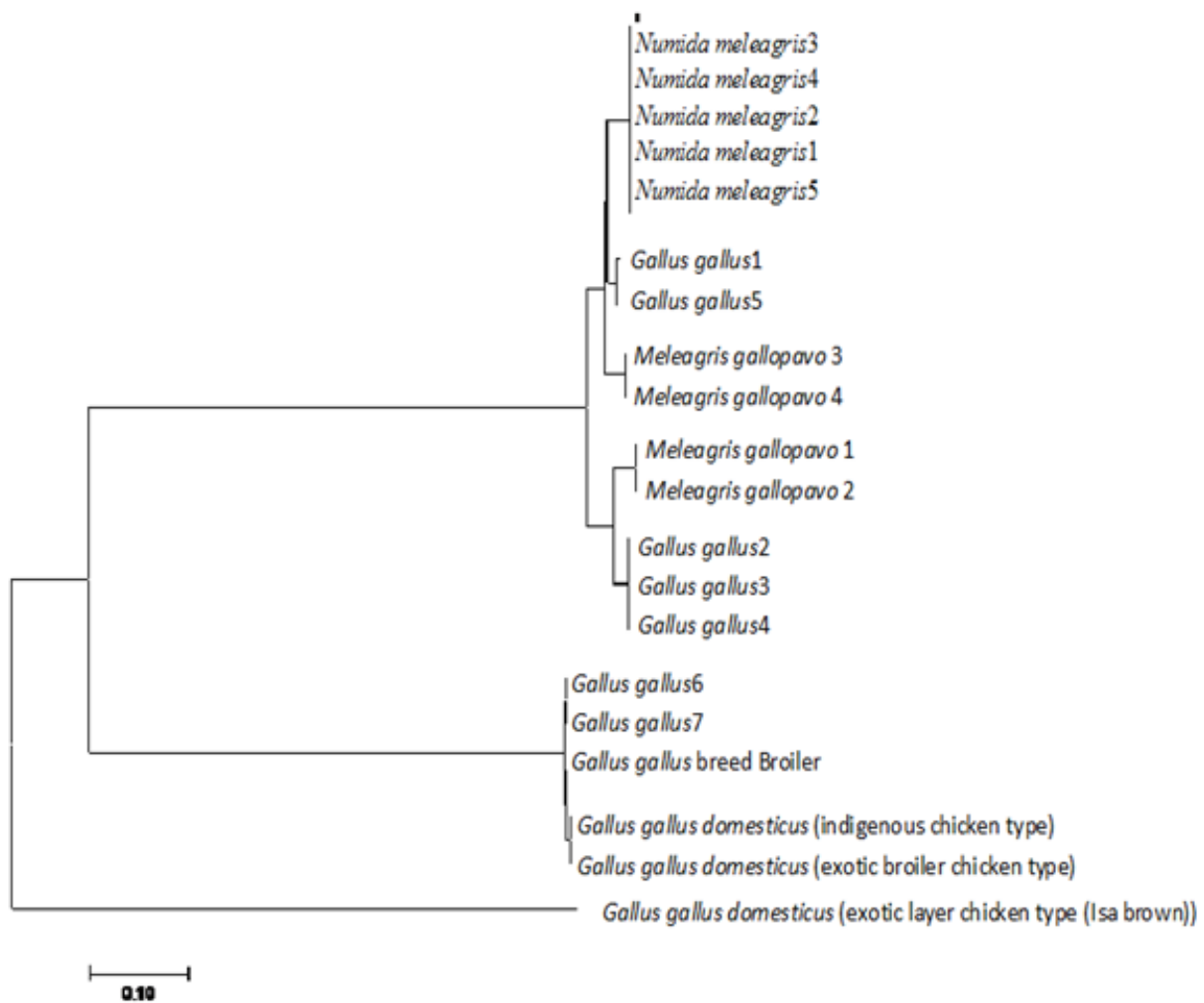


Figure 1. A Phylogenetic Tree Based on Nucleotide Sequence Variation in the *NRAMP1* Gene

The phylogenetic tree that was based on the *NRAMP1* gene fragment nucleotide sequence revealed that both the indigenous and exotic broiler chicken types are genetically similar; both were in the same node of a cluster, but the exotic layer chicken type was completely separate from all other breed and poultry species, including the helmeted guinea fowl (*Numida meleagris*), the turkey (*Meleagris gallopavo*) and chickens (*Gallus gallus*) of both the exotic and the indigenous types. However, the gene appears to vary within the *Gallus* breeds or types because different clusters with groups of *Gallus* were revealed. Furthermore, the gene was revealed to be similar within species, as shown by different species in separate clusters (Figure 1).

## Discussion

The *NRAMP1* gene fragment, when amplified, was non-polymorphic in both indigenous and exotic chicken types, which indicated the presence of only one homozygous allele. Indigenous chickens have been reported to have a higher frequency of the “resistant allele” than

exotic birds do [20]. Lamont *et al.* [3] and Lamont [21] reported that polymorphism in the *NRAMP1* gene is implicated with a *Salmonella enteritidis* (SE) response in different breeds of chickens; however, some line broiler sires with specific alleles of the *NRAMP1* gene showed improved resistance to the pathogen. Girard-Santosuosso *et al.* [13] showed that genetic variations exist in the resistance to pathogenic infection in some exotic flocks, which can be attributed in part to genetic polymorphism in the *NRAMP1* region. Muhsinin *et al.* [22] also reported that the *NRAMP1* was polymorphic among the native chickens that they studied. They added that the homozygous genotype CC was predominant within their population. These findings indicate that environment/location may play a significant role in determining the genotypes that are observed within the population.

However, nucleotide sequence analysis of the gene revealed that SNP showed variations, with some amino acid changes between the indigenous and exotic breeds. The SNP substitutions include C to T, G to C, A to C,

and C to G. These substitutions may have a significant association with disease resistance in these chicken types. Different SNPs have been associated with different traits in poultry. These are indications that SNPs play a significant role in gene expression and consequently the manifestation of either the protein or the trait [13, 23, 24]. Genetic relatedness that has been observed between indigenous chicken and exotic broiler chicken sequences may suggest the possession of a similar resistance ability to bacterial and viral infections that is different from the layer chicken breed, thereby implicating nucleotide polymorphism in this gene as a crucial genetic factor in the poultry's innate disease resistance ability. Liu *et al.* [4] reported no significant effect on the SNP variation among the studied population, although they reported that allele C cleared the infection better than did allele T. Tohidi *et al.* [10] reported polymorphism in the *NRAMP1* gene with significant association with clearing the SE burden in the studied poultry population. Kramer *et al.* [25] also reported that the homozygous C/C genotype in the *NRAMP1* gene was related to the highest SE load. It is important to note that there is inconsistency among the results of different studies about the variations and the effect on different poultry populations. This may be a result of different breeds, lines, or types responding differently to different infections. It may also be due to the effect of some SNPs being either silent and/or linked to another gene sequence nearby. The *NRAMP1* plays an important role in innate immunity against infections [26, 27]. According to Tohidi *et al.* [10], the *NRAMP1* gene has strong potential in disease resistance. Hence, the gene may play a significant role in selection programs for increasing genetic resistance against diseases in poultry.

The phylogenetic tree that was based on the nucleotide sequence of the *NRAMP1* gene revealed that the different poultry species branched out to form separate clusters. The exotic layer chicken type in general showed high genetic distance from the indigenous, exotic broiler, and other poultry species. The indigenous and exotic broiler chicken types showed closer genetic relatedness than they did with the exotic layer chicken type. This indicates that there is a higher homology between the indigenous and exotic chicken types. Furthermore, the indigenous and exotic broiler chicken types also showed more genetic relatedness with the guinea fowl (*Numida meleagris*) and turkey (*Meleagris gallopavo*) than with the exotic layer. Girard-Santosuosso *et al.* [13] showed that differences between populations in response to infections may be a result of different genetic structures in the different populations of the poultry types that were studied. Hu *et al.* [1] reported that the genotype of the *NRAMP1* gene in individual populations or species plays a significant role in their overall disease resistance. However, the gene is similar within species and may vary between and/or among breeds.

## Conclusion

As shown in this study, variations in the gene may play a significant role in the way that each breed or species responds to different infections. Hence, the gene may be a potential selection molecular marker for developing indigenous hybrids that will possess the desired traits of resistance to poultry pathogenic infections. Furthermore, extensive studies are necessary to evaluate heritability, polymorphism's effect on gene expression, and the molecular mechanism that is caused by polymorphisms within the gene among the different poultry species. Breeding with the aim of improving natural resistance ability may not completely prevent infectious disease occurrence or outbreaks in poultry; however, through marker-assisted selective breeding, improved innate resistance ability can reduce the morbidity and economic losses that are caused by poultry infectious diseases globally. In view of the necessity for rapid improvement of the productivity capacity of poultry, it is pertinent to note that exploration and improvement of the innate disease resistance characteristics may be of greater economic value to the poultry industry and global public health than is the use of antibiotics.

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## Competing Interests

The authors have declared that no competing interests exist.

## Authors Contributions

Authors UDA, KOA, and BOO designed the study. UDA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. KOA and BOO performed supervisory roles. Author UDA managed the analyses of the study. Author UDA managed the literature searches. All authors read and approved the final manuscript.

## References

- [1] Hu, G.S., Chang, G.B., Zhang, Y., Hong, J., Liu, Y., Chen, G.H. 2011. Association Analysis Between Polymorphisms of *Nramp1* Gene and Immune Traits in Chicken. *J. Anim. Vet. Adv.* 10(9): 1133-1136.
- [2] Hu, J., Bumstead, N., Skamene, E., Gros, P., Malo, D. 1996. Structural Organization, Sequence, and

- Expression of the Chicken NRAMP1 Gene Encoding the Natural Resistance-Associated Macrophage Protein 1. *DNA Cell. Biol.* 15(2): 113-23, <https://doi.org/10.1089/dna.1996.15.113>.
- [3] Lamont, S.J., Kaiser, M.G., Liu, W. 2002. Candidate genes for resistance to *Salmonella enteritidis* colonization in chickens as detected in a novel genetic cross. *Vet. Immunol. Immunop.* 87(3-4): 423-428, [https://doi.org/10.1016/S0165-2427\(02\)00064-8](https://doi.org/10.1016/S0165-2427(02)00064-8).
- [4] Liu, W., Kaiser, M., Lamont, S.J. 2003. Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with *Salmonella enteritidis* in young chicks. *Poult. Sci.* 82: 259-266, <https://doi.org/10.1093/ps/82.2.259>.
- [5] Wigley, P. 2004. Genetic resistance to *Salmonella* infection in domestic animals. *Res. Vet. Sci.* 76(3): 165-169. [https://doi.org/10.1016/S0034-5288\(03\)01117-6](https://doi.org/10.1016/S0034-5288(03)01117-6).
- [6] Puzyrev, V.P., Freidin, M.B., Rudko, A.A., Strelis, A.K., Kolokolova, O.V. 2002. Polymorphisms of Tuberculosis Susceptibility Candidate Genes in the Slavonic Population of Siberia: A Pilot Study. *Mol. Biol.* 36(5): 634-636. doi: 10.1023/A:1020611111205.
- [7] Stagas, M.K., Papaetis, G.S., Orphanidou, D., Kostopoulos, C., Syriou, S., Reczko, M., Drakoulis, N. 2011. Polymorphism of the NRAMP1 gene: Distribution and susceptibility to the development of pulmonary tuberculosis in the Greek population. *Med. Sci. Mon. Int. Med. J. Exp. Clin. Res.* 17(1): PH1-PH6.
- [8] Mazzolla, R.A., Manuela, P.A., Roberta, B.A., Rachele, N.B., Francesco, B.A., Giuseppe, B.C., Blasi, E. 2002. Differential microbial clearance and immunoresponse of Balb/c (NRAMP1 susceptible) and DBA2 (NRAMP1 resistant) mice intracerebrally infected with *Mycobacterium bovis* BCG (BCG). *FEMS Immunol. Med. Microbiol.* 32: 149-158. <https://doi.org/10.1111/j.1574-695X.2002.tb00547.x>.
- [9] Soe-Lin, S., Sameer, S.A., Andriopoulos, B., Andrwa, M.C., Schranzhofer, M., Kahawita, T., Gaarcia-Santos, D., Ponka, P. 2009. NRAMP1 promotes efficient macrophage recycling of iron following erythrocytosis in vivo. *PNAS USA.* 106(14): 5960-5965. <https://doi.org/10.1073/pnas.0900808106>.
- [10] Tohidi, R.I., Idris, B.I., Panandam, J.M., Bejo, M.H. 2013. The effects of polymorphisms in 7 candidate genes on resistance to *Salmonella Enteritidis* in native chickens. *Poult. Sci.* 92(4): 900-909. <https://doi.org/10.3382/ps.2012-02797>.
- [11] Marquet, S., Lepage, P., Hudson, T.J., Musser, J.M., Schurr, E. 2000. Complete nucleotide sequence and genomic structure of the human NRAMP1 gene region on chromosome region 2q35. *Mamm. Genome.* 11(9): 755-62. doi: 10.1007/s003350010151.
- [12] Klug, W., Cummings, M., Spencer, C., Palladino, M. 2009. *Concept of genetics*. 9th ed. New York: Person International Publication. p776
- [13] Girard-Santosuosso, O., Lantier, F., Lantier, I., Bumstead, N., Elsen, J.M., Beaumont, C. 2002. Heritability of susceptibility to *Salmonella enteritidis* infection in fowls and test of the role of the chromosome carrying the NRAMP1 gene. *Genet. Sel. Evol.* 34:211. doi: 10.1051/gse:2002004.
- [14] Afolabi, K.D. 2013. Sustainable Food Security in the Era of Local and Global Environmental Change. In: Mohamed B, Olaf P, Gabrielle K, editors. *Local or Indigenous Chicken Production: A Key to Food Security, Poverty Alleviation, Disease Mitigation and Socio-Cultural Fulfillment in Africa*. p217-229 doi: 10.1007%2F978-94-007-6719-5
- [15] FAO. 2010. Chicken genetic resource used in smallholder production systems and opportunities for their development, by P Sorensen. FAO Smallholder poultry production paper No. 5. Rome, Italy. <https://www.forskningsdatabasen.dk/en/catalog/2389309643>.
- [16] Pym, R. 2010. Genetic diversity and conservation of genetic resources. FAO Poultry development review: Poultry genetics and breeding in developing countries. <http://www.fao.org/3/a-al728e.pdf>.
- [17] Zanetti, E., Dalvit, C., De-Marchi, M., Zotto, R., Cassandro, M. 2007. Genetic characterisation of Italian chicken breeds using a panel of twenty microsatellite markers. *Poljoprivreda.* 13(1): 1-5, [https://hrcak.srce.hr/index.php?show=clanak&id\\_clanak=16137](https://hrcak.srce.hr/index.php?show=clanak&id_clanak=16137).
- [18] Singh, D.P., Fosta, A.J and Thieme O. 2011 Summary and conclusions of the first e-conference of the international network for family poultry development (INFPD) on the theme "Opportunities for poultry breeding programmes for family production in developing countries: The bird for the poor" FAO, Rome, Italy.
- [19] Sambrook, J., Fritschi, E.F., Maniatis, T. 1989. *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, New York. <http://www.fao.org/3/a-al728e.pdf>.
- [20] Fulton, J.E., Arango, J., Ali, R.A., Bohorquez, E.B., Lund, A.R., Ashwell, C.M., Settler, P., O'Sullivan, N.P., Koci, M.D. 2014. Genetic Variation within the Mx Gene of Commercially Selected Chicken Lines Reveals Multiple Haplotypes, Recombination and a Protein under Selection Pressure. *PLoS ONE.* 9(9): e108054. <https://doi.org/10.1371/journal.pone.0108054>.
- [21] Lamont, S.J., Pinard-van der Laa, M.H., Cahaner, A., Van der Poel, J.J. Parmentier H.K. 2003. Selected for resistance: direct selection on the immune response. In: Muir W.M., Aggrey S.E., edi-

- tors. Poultry genetic, breeding and biotechnology. Oxford: CAB International. 399-418.
- [22] Muhsinin, M., Ulupi, N., Gunawan, A., Wibawan, W.T., Sumantr, C. 2016. Association of NRAMP1 Polymorphisms with Immune Traits in Indonesian Native Chickens. *Int. J. Poult. Sci.* 15(10): 401-406. doi: 10.3923/ijps.2016.401.406.
- [23] Lei, M., Luo, C., Peng, X., Fang, M., Nie, Q., Zhang, D., Yang, G., Zhang, X. 2007. Polymorphism of Growth-Related Genes Associated with Fatness and Muscle Fiber Traits in Chickens. *Poult. Sci.* 86: 835-842. <https://doi.org/10.1093/ps/86.5.835>.
- [24] Khoa, D.V., Khang, N.T., Ngu, N.T., Matey, J., Loan, H.P., Thuy, N.D. 2013. Single Nucleotide Polymorphisms in Gh, Ghr, Ghsr and Insulin Candidate Genes in Chicken Breeds of Vietnam. *Greener J. Agric. Sci.* 3(10): 716-724.
- [25] Kramer, J., Malek, M., Lamont, S.J. 2003. Association of Twelve Candidate Gene Polymorphisms and Response to Challenge with *Salmonella* Enteritidis in Poultry. *Animal Genet.* 34(5):339-348. <https://doi.org/10.1046/j.1365-2052.2003.01027.x>.
- [26] Gruenheid, S., Pinner, E., Desjardins, M., Gros, P. 1997. Natural resistance to infection with intracellular pathogens: The NRAMP1 protein is recruited to the membrane of the phagosome. *J. Exp. Med.* 185: 717-730. doi: 10.1084/jem.185.4.717.
- [27] Berndt, A., Wilhelm, A., Jugert, C., Pieper, J., Sachse, K., Methner, U. 2007. Chicken cecum immune response to *Salmonella enterica* serovars of different levels of invasiveness. *Infect. Immunol.* 75:5993-6007. doi: 10.1128/IAI.00695-07.