EVALUATION OF BREED EFFECT ON GENE EXPRESSION OF ARYL HYDROCARBON RECEPTOR (AHR), CYTOCHROME P450 (CYP1A1) AND INTERLEUKINS (IL2) IN CHICKENS REARED IN A CRUDE OIL-POLLUTED ENVIRONMENT

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ABSTRACT

Hydrocarbon toxicity resulting from the disruption of homeostasis caused by exposure to hydrocarbon pollutants is a significant obstacle that could impede the expression of the full genetic potentials of chicken breeds in the Niger Delta. Depending on the population, the mechanism and severity of toxicity may vary with the level of expression of certain genes modulated by xenobiotics. This study investigated breed effects on expression of aryl hydrocarbon receptor (AhR), cytochrome p450 (CYP1A1) and interleukins (IL2) in chickens. For the investigation, two breeds of chicken: (Nigerian native chickens and commercial line broiler chickens) raised under extensive system by rural smallholder farmers in Okrika and Nsukka Local government Areas, Rivers and Enugu States, Nigeria were utilized. Liver tissue was excised from individual bird (27 birds per breed) for RNA extraction and gene expression studies. Results revealed that there was significant breed effect in the response of chickens in Okrika where the expression of AhR, CYP1A1 and IL-2 genes was upregulated in the native chickens ($p<0.05$). Such disparities were not observed in chickens reared in Nsukka. This could be an indication of increased metabolism and detoxification of hydrocarbon pollutants in the native chicken particularly in hydrocarbon polluted environment. We therefore propose studies on AhR, CYP1A1 and IL-2 as molecular markers for evaluating hydrocarbon toxicity in chickens and possible selection for genetic improvement.

Keywords: aryl hydrocarbon receptor, cytochrome p450, chickens, interleukins, hydrocarbon pollution, oxidative stress biomarkers

INTRODUCTION

Environmental pollution from oil spillage, natural gas flaring and organic pollutants is ubiquitous in the Niger Delta region of Nigeria due to crude oil exploration (Chukwumati and Asiegbu, 2023). Hydrocarbon toxicity poses a great threat not only to the mangrove and aquatic ecosystem in this region but also to the livestock sector especially the fowl which contributes essentially to the socioeconomic status of the rural households. The major breeds commonly reared are the Nigerian native (indigenous) chickens, and broiler chickens. These birds are exposed to hydrocarbon pollutants through ingestion of contaminated food products/water and inhalation of gaseous pollutants. Existence of disparity in the mechanism and level of tolerance to hydrocarbon fragments among species was highlighted by Karchner *et al.* (2006) and Bianchini and Morrissey (2020). We, therefore, hypothesize that possible variations in hydrocarbon toxicity tolerance levels in these chicken breeds could influence their well-being and productivity. Meanwhile, there appears to be a paucity of information on the role of gene modulated by xenobiotics, such as hydrocarbon pollutants, in poultry. These receptors associate environmental chemical stimuli with immune response, cellular homeostasis or adaptive response (Esser, 2012). Modulation of the immune system of chickens was reported by Wlaźlak *et al.* (2023) to be a vital factor in poultry production. Thus, improving adaptability to hydrocarbon tolerance to enhance productivity and well-being of these birds is critical (Oleforuh-Okoleh *et al.,* 2023). In the present study we investigated three receptors, aryl hydrocarbon receptor (AhR), cytochrome p450 (CYP1A1) and interleukins (IL2) in chickens reared in Nsukka and Okrika Local Government Areas in Rivers and Enugu states of Nigeria. crude oil-polluted communities.

MATERIALS AND METHODS

The study was conducted on Nigerian native chickens (light ecotype according to the classification of Momoh et al., 2008) and broiler chickens (mixed strains) reared in Okrika and Nsukka Local Government Areas of Rivers and Enugu States, Nigeria, respectively. Okrika hosts many petrochemical activities than Nsukka. Nsukka was considered as the control group. The chickens used for the study were raised under extensive system of management, and were obtained from rural smallholder farmers. The chickens were humanely sacrificed and about 350mg of liver tissue excised from individual bird (54 birds, 27 per breed), and preserved in RNA later to prevent nucleic acids degeneration. RNA was extracted using procedures including the cell lysis, RNA binding, RNA washing and RNA elution. The isolated RNA was converted to cDNA which was amplified by Real-Time PCR Detection with forward and reverse primers of the following genes: AhR, CYP1A1 and IL-2 with Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeper gene (Table 1) by modifying of procedures described by Sikiru *et al.* (2019). The cycling conditions included an initial activation at 95 °C for 12 minutes, denaturation at 95 °C for 15 seconds, annealing, and elongation at 72 °C for 20 seconds, respectively. All genomic analysis was done at The genomic analysis was done at African Biosciences (JaaGee House 88, Km 6 Ibadan-Ife Expressway, Olade, Ibadan, Oyo state, Nigeria)

Table 1. List of the target genes for expression analysis and their respective primers details

Gene name	Accession number	Primer Type	Primer sequence	Primer length
Aryl-hydrocarbon receptor (AHR1A)	NM_204118.3	F	CGGAAACCTGTGCAGAAAATAGT	20
		R	AAAAACCAGAGCATCTGCCG	
Interleukin 2 (IL-2)	NM_204153.2	F	TCTCGAGCTCTACACCCCAA	20
		R	CCGGTGTGATTTAGACCCGT	
Cytochrome P450 (CYP1A1)	NM_205147.2	F	GTGATGGAGGTGACCATCGG	20
		R	ATTCGTAGCTGAACGCCAGG	
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_204305.2	F	TCAAATGGGCAGATGCAGGTGATGGCATGGACA	20
		R	GTGGTCAGATGGCATGGACAGTGGTCA	20

Data obtained were subjected to differential expression analysis (fold change) and independent sample t- test. Mean values were considered significant at adjusted p-value <0.05.

RESULTS AND DISCUSSION

The fold change result from the RT-PCR analysis indicated that the three genes studied (AhR, CYP1A1 and IL2) were significantly differentially expressed ($p<0.05$) with the native chickens showing more expression compared to the broilers (Figure 1) in Okrika though in Nsukka no differential expression was obtained in both breeds and the expression was down-regulated (Figure 2). There are several evidences that AhR mediates the toxic responses associated with environmental pollutants such as polycyclic aromatic hydrocarbons, and particulate matters (Goode, et al., 2014; Dietrich, 2016) found in crude oil exploration sites. The current finding affirms studies which demonstrated that activation of the AhR led to increased induction of target xenobiotic metabolism enzymes like CYP1A1 (Vogel *et al.*, 2020; Noda et al., 2023). AhR activation was also found to increase the expression of genes involved in immune regulation such as IL-2 (Ehrlich *et al.,* 2017). IL-2 is essential for the development of T regulatory cells thereby mediating tolerance while reducing inapt immune reactions (Liao *et al.,* 2013).

The upregulation in the candidate genes in Okrika implies that there was positive feedback upon the environmental assault. In other words, more receptor proteins would have been synthesized and transported to the membrane of the cell bringing back the sensitivity of the cell to normal thereby reestablishing homeostasis. Thus, the increased expression of these genes may lead to increased metabolism and detoxification of PAHs, which can help to reduce the toxic effects of these compounds and modulating the immune response in health and disease. Li *et al.* (2022) noted that the presence of upregulated genes in an organism may help it to adapt to the influence of "genomic shock" and cope with the natural environment. Therefore, the upregulation of these genes in native

chickens compared to broiler chickens in Okrika suggests that the native chicken population studied may be more adapted to living in environments with elevated levels of hydrocarbon pollution.

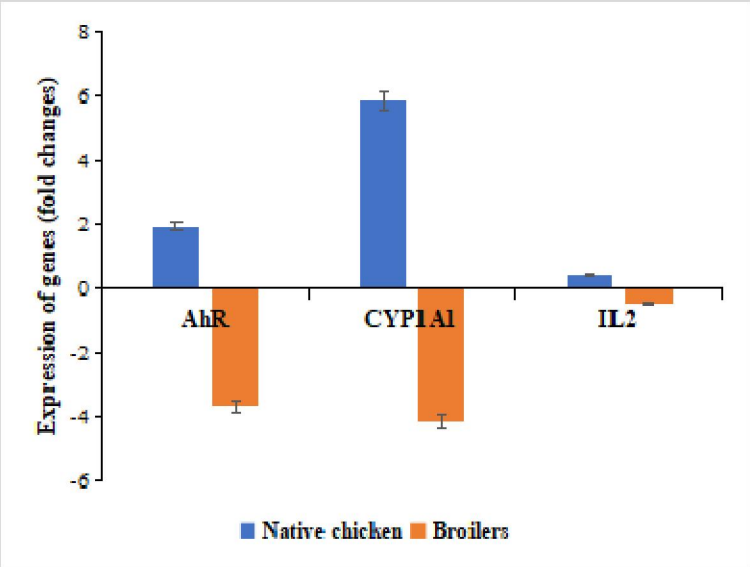

Figure 1: Shown are the mean fold change of the relative expression of AhR, CYP1A1 and IL-2 in liver tissues of the native and broiler chickens reared in Okrika. Okrika hosts lots of petrochemical industries. The upregulation of the AhR and CYP1A1 in the native chicken is an indication that they may have higher adaptive response to hydrocarbon pollutant than the broilers.

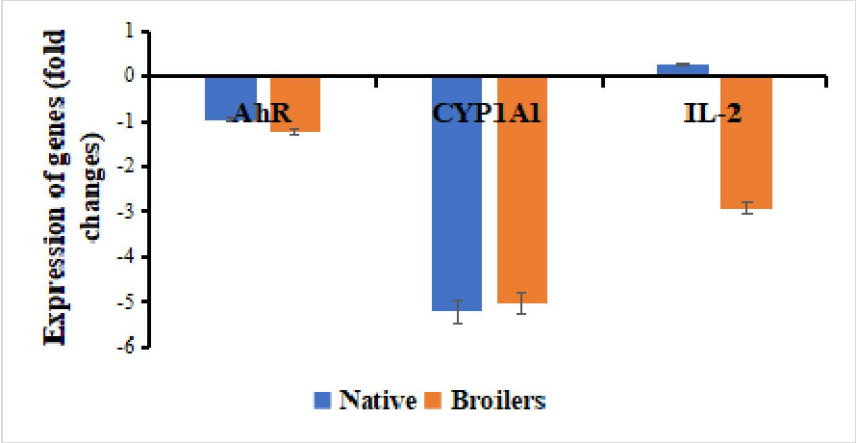

Figure 2: Relative expression of AhR, CYP1A1 and IL-2 in liver tissues of the native and broiler chickens reared in Nsukka. Nsukka is not predisposed to petrochemical activities as Okrika. The downregulation of the AhR and CYP1A1 in both strains implies that the birds raised may not have been exposed so much to hydrocarbon pollutants to trigger the activation of the xenobiotic enzymes.

Conclusion

The study revealed that exposure of chickens to hydrocarbon polluted environment had significant impact on the immune function and xenobiotic metabolism in chickens especially the native chickens. Long exposure period of the native chickens to the environment could have also contributed to the observations made. We therefore suggest further studies of these breeds under controlled exposure. Such studies would aid in understanding the mechanisms involved in the expression of these genes in response to environmental pollutants. There could also be association studies of these genes with production traits to elucidate economic implications and possibly initiate selection towards genetic improvement of the available breeds using these genes as molecular markers.

REFERENCES

Azeez, O. M., Akhigbe, R. E., Anigbogu, C. N. 2013. Oxidative status in rat kidney exposed to petroleum hydrocarbons. *J Nat Sci Biol Med.* Jan;4(1):149-54. doi: 10.4103/0976-9668.107280. PMID:23633852; PMCID: PMC3633267.

Bianchini, K. and Morrissey, C. A. (2020). Species traits predict the aryl hydrocarbon receptor I (AHRI) subtypes responsible for dioxin sensitivity in birds. Sci. Rep 10, 11706. doi:10.1038/s41598.020.68497-y.

Chukwumati, J. A. and Asiegbu, G. C. 2023. Accumulation of hydrocarbons and some heavy metal contents on sediments and plants from crude oil mangrove ecosystem in Okirika, Nigeria. *WJARR.* 17(03), 298-306. doi.ord/10.30574/wjarr.2023.17.3.0999,

Ehrlich, A. K., Pennington, J. M., Tilton, S., Wang, X., Marshall, N. B., Rohlman, D., Funatake, C., Punj, S., O'Donnell, E., Yu, Z., Kolluri, S.K., Kerkvliet, N.I. 2017. AhR activation increase IL-2 production by alloreactive CD4+ T cells initiating the differentiation of mucosal-homing Tim3+ Lag3+ Tr1 cells. *Eur J Immunol.* 47(11):1989-2001. doi: 10.1002/eji.201747121. PMID: 28833046; PMCID: PMC5927372.

Esser, C. 2012. biology and function of aryl hydrocarbon receptor:report of an international and interdisciplinary conference. *Arch. Toxicol.* Aug:86(8): 1323-9. doi: 10.1007/s00204-012-0818-2. PMID: 22371237.

Goode, G., Pratap, S., Eltom, S. E. 2014. Depletion of the aryl hydrocarbon receptor in MDA-MB-231 human breast cancer cells altered the expression of genes in key regulatory pathways of cancer. *PLoS One*. 2014 Jun 16;9(6):e100103. doi: 10.1371/journal.pone.0100103.

Karchner, S. I., Franks, D. G., Kennedy, S. W., and Hahn, M. E. 2006. The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6252–6257. doi:10.1073/pnas.0509950103

Li, M., Hu, M., Xiao, Y., Wu, X., Wang, J. 2002. The activation of gene expression and alternative splicing in the formation and evolution of allopolyploid Brassica napus. *Hortic Res*. 2022 Jan 19;9:uhab075. doi: 10.1093/hr/uhab075. PMID: 35043208; PMCID: PMC8923814.

Liao, W., Lin J-XX, and Leonard, W. J. 2012. interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity* 38. 13 -25. doi.org/10.1016/j.immuni.2013.01.004.

Momoh, O. M. and Nwosu, C. C. 2008. Genetic evaluation of growth traits in crosses between two ecotypes of Nigerian local chicken Livestock Research for Rural Development. 20(10). http:www.Irrd.org/Irrd20/10/momo20158.htm.

Noda, S., Harada, N., Hida, A., Fujii-Kuriyama, Y., Motohashi, H., Yamamoto, M. 2003. Gene expression of detoxifying enzymes in AhR and Nrf2 compound null mutant mouse, *Biochemical and Biophysical Research Communications*. 303(1) 105-111, https://doi.org/10.1016/S0006-291X(03)00306-1.

Oleforuh-Okoleh, V. U., Sikiru, A. B., Kakulu, I. I., Fakae, B. B., Obianwuna, U. E., Shoyombo, A. J., Adeolu, A. I., Ollor, O. A. and Emeka, O. C. 2023. Improving hydrocarbon toxicity tolerance in poultry: role of genes and antioxidants. *Front. Genet.* 14:1060138. doi: 10.3389/fgene.2023.1060138

Sikiru, A. B., Arangasamy, A., Alemede, I. C., Guvvala, P. R., Egena, S. S. A., Ippala, J. R., and Bhatta, R. (2019). *Chlorella vulgaris* supplementation effects on performances, oxidative stress and antioxidant genes expression in liver and ovaries of New Zealand White rabbits. *Heliyon*, *5*(9), e02470. https://doi.org/https://doi.org/10.1016/j.heliyon.2019.e02470

Vogel, C. F. A., Van Winkle, L. S., Esser, C., Haarmann-Stemmann, T. 2020. The aryl hydrocarbon receptor as a target of environmental stressors - Implications for pollution mediated stress and inflammatory responses. *Redox Bio*l. Jul; 34:101530. doi: 10.1016/j.redox.2020.101530. . PMID: 32354640; PMCID: PMC7327980.

Wlaźlak, S., Pietrzak, E., Biesek, J., Dunislawska, A. 2023. Modulation of the immune system of chickens a key factor in maintaining poultry production-a review. *Poult Sci.* 102(8):102785. doi: 10.1016/j.psj.2023.102785. PMID: 37267642; PMCID: PMC10244701.