Expression Analysis of Orexin Gene in Two Nigerian Indigenous and Exotic Chickens Using Quantitative Polymerase Chain Reaction (qPCR)

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Abstract
The Nigerian Indigenous Chickens are suitable for development of meat strain for tropical environment; characterized by small body size and slow growth. The orexin gene serves as a crucial regulator of appetite, energy balance, and stress responses in poultry and important molecular marker for understanding breed-specific physiological adaptations. This study investigated orexin gene expression patterns in two Nigerian Indigenous Chickens which are Fulani and Yoruba ecotypes and exotic; Cobb-500 broilers using quantitative Polymerase Chain Reaction (qPCR) technology. Total of 135 birds (45 per breed) were reared for four weeks, after which liver tissue samples were collected for RNA extraction and analysis. Ribonucleic Acid (RNA) was extracted using the Zymo RNA mini prep kit, followed by cDNA synthesis and qPCR analysis using Luna® Universal qPCR Mastermix. The TATA box binding protein served as the housekeeping gene for normalization. Gene expression was quantified using the 2^-ΔΔCT method (Livak method) to determine fold changes between breeds. Results revealed significant inter-breed variations in orexin expression levels ($P < 0.05$). The Fulani chickens demonstrated higher expression (1.37-fold), then Cobb-500 broilers (0.35-fold), while Yoruba ecotype chickens had lower expression (0.02-fold). Melt curve analysis confirmed primer specificity and amplification consistency across all samples. The findings suggested that elevated orexin expression in Fulani chickens may reflect superior physiological adaptability and energy regulation capabilities, supporting their resilience in variable environmental conditions. The differential expression patterns highlight orexin's potential as a molecular marker for selective breeding programs aimed at improving indigenous chicken productivity while maintaining genetic diversity and environmental adaptability.

Keywords: Orexin, DNA, RNA, qPCR, Nigerian indigenous chickens

DESCRIPTION OF PROBLEM

Poultry farming in Nigeria holds a significant position in the agricultural landscape, providing a vital source of nutrition and livelihood for millions of people (1). The poultry agro-business in Nigeria is the most commercialized among all agricultural sub-sectors and has contributed massively to Nigeria's economy (2). Poultry farming in Nigeria accounts for over 180 million birds yearly and yields an annual production of approximately 454 billion tonnes of meat and 3.8 million eggs (3). Among the diverse poultry population in Nigeria, indigenous chickens constitute a significant majority, approximately 80 percent of the chicken population in rural areas in Nigeria (4). The Fulani and Yoruba eco type chickens represent the most popular and distinct breeds of Nigerian indigenous chickens, each with unique characteristics deeply rooted in the country's cultural and agricultural heritage. Understanding the physiological factors and mechanisms of these chickens is of utmost importance to animal nutrition and biotechnology. The Fulani chickens originate from the tough and weathered regions of Nigeria. The Fulani people who raise them live in small groups and maintain genetic isolation, which prevents interbreeding with other native chickens (5). The Yoruba ecotype has drawn attention due to its potential as an egg-type chicken, attributed to its distinctive morphology (6). This ecotype exhibits high genetic variation, indicating its ability to cope with environmental changes and stressors (7). The breed has proven its resilience by thriving in backyard systems across villages, towns, and cities, showcasing remarkable adaptation to stressful and harsh conditions (8).

MATERIALS AND METHODS

Experimental Site

The experimental aspect was carried out at the Layers Unit, Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso. Ogbomoso is located in the derived Savanna Zone that lies on longitude 4°10′ East of Greenwich Meridian and latitude 8°10′ North of the equator. The altitude ranges

from 300m and 600m above sea level while mean temperature and annual rainfall are 27°C and 1247mm respectively (9).

The laboratory aspect of the study was carried out in Acutig Genetic Laboratory, behind MS house, Asero Estate, Abeokuta. The laboratory region receives an annual rainfall ranging from 1,270 mm to 1,800 mm, with temperatures typically between 22°C and 33°C. The relative humidity is generally high, averaging around 80%. It lies between latitude 7° 10′ N and 7° 15′ N and longitudes 3° 17′ E and 3° 26′ E (10).

Experimental Birds and their Management

For this study, 45 birds each from the Fulani and Yoruba ecotypes of Nigerian Indigenous Chickens, and the exotic chicken (Cobb-500) were procured at 4 weeks of age, totaling 135 birds. The birds were chosen from diverse geographic locations representing the respective ecotypes. The birds were reared over a period of 4 weeks and fed without restriction for the duration of the experiment.

RNA Extraction Procedure

Tissue Collection

After the experiment, tissue samples, specifically liver, were collected from the selected birds after slaughtering. Samples were immediately stored in RNA later to preserve RNA integrity. Storage with RNA later is essential to preserve the integrity of RNA, as RNA is susceptible to degradation by ribonucleases, which can become active when tissues are exposed to higher temperatures. High-quality RNA is critical for accurate gene expression analysis.

RNA Extraction

Total RNA was extracted from the liver tissues using the Zymo RNA mini prep kit, following the manufacturer's instructions.

Sample Preparation

Tissue of 25mg was submerged in 600µl of RNA lysis buffer, centrifuged at 14000g (relative centrifugal force) for 30s, and the supernatant was transferred into a nuclease-free tube.

Total RNA Extraction

All procedures were performed at room temperature, as room temperature enhances cell lysis and protein denaturation, critical for releasing intact nucleic acids (11). The content from the nuclease free tube was transferred into the Spin-Away™ filter in a collection tube and centrifuged at 16,000g for 30s. This is to remove majority of the genomic DNA to the flow through. 600µl of 96% ethanol was added and mixed well by vortexing. The mixture was then transferred into the Zymo-Spin IIICG1 column in a collection tube and centrifuged at 16000g for 30s. The flow through was discarded.

DNase I Treatment

The column was then washed with 400µl RNA Wash buffer and centrifuged at 16000g for 30s, the flow through was discarded. 5µl DNase I, 75µl DNA digestion buffer were added into a nuclease free tube, mixed and the mixture was then added directly into the column matrix and incubated for 15min at 30°C. 400µl of RNA prep buffer was added to the column after incubation, centrifuged and the flow through was discarded. 700µl of RNA wash buffer was added to the column and centrifuged at 16000g for 30s and the flow through was discarded. 400µl of RNA wash buffer was added and allowed to stand for about 60s to ensure complete removal of the wash buffer. The column was then transferred into a nuclease free tube. 100µl of DNase/RNase-free water was added directly to the column matrix and centrifuged at 14000g for 30s. The flow through was stored for conversion to cDNA.

Complementary Deoxyribonucleic Acid (cDNA) Synthesis

Complementary DNA was synthesized using the LunaScript® RT SuperMix kit. A total reaction volume of 20µl was prepared according to the manufacturer's instructions.

The components were prepared according to Table 1.

After preparations, the set up was then run in the thermal cycler using the conditions in Table 2.

Quantitative Polymerase Chain Reaction (qPCR)

Quantitative Polymerase Chain Reaction assay was performed using the Luna® Universal qPCR Mastermix (M3003), following the manufacturers' protocol. A qPCR cocktail was first prepared, before being aliquoted into 20µl reaction volumes and run in the qPCR machine. The cocktail was prepared by adding all the components of the master mix except the template as seen in Table 3.The primers used for this work was the OREX primer and the TATA box BP as the house keeping gene as presented in Table 4.

Statistical Analysis

Livak method (2^-ΔΔCT) was used to analyze fold change, which is the level of expression of the Orexin

gene in the different chicken breeds, and means were separated using Duncan's multiple range test (12).

RESULTS AND DISCUSSION

The melt curve confirmed specific amplification of the orexin gene across all samples. Individual melt curve peaks showed minimal variation, with the highest fluorescence intensity (FI) recorded in a Cobb-500 sample (84.67 FI) and the lowest in a Yoruba ecotype sample (81.82 FI). The melt curve analysis confirmed the specificity and consistency of amplification across all samples. Minor variation in fluorescence intensity among breeds likely reflects breed-specific genetic differences in the orexin gene target region. The average melt curves were nearly identical across the three groups, indicating stable amplification conditions. This uniformity supports the technical reliability of the qPCR data, reducing the likelihood of primer-dimer formation or nonspecific amplification. Fold change analysis using the 2^-ΔΔCT method revealed clear inter-breed differences. Yoruba ecotype chickens showed the lowest orexin expression, averaging a fold change of 0.02. Cobb-500 broilers showed moderate expression (0.35). Fulani ecotype chickens recorded the highest expression level (1.37). These differences were statistically significant ($P < 0.05$) and suggest that orexin expression is not uniform across breeds. The low expression in Yoruba chickens may be associated with lower appetite stimulation or reduced energy mobilization. This supports earlier findings that indigenous breeds often show reduced growth potential under controlled conditions due to their evolutionary adaptation to low-input systems (13). In contrast, the Fulani ecotype, with the highest orexin expression, may be better adapted for active foraging, alertness, and physiological resilience. This pattern aligns with studies linking orexin signaling to metabolic activity and behavioral responsiveness (14). (15) revealed that orexin regulates hepatic lipogenesis in chickens, reinforcing its role in energy homeostasis.

Table 1: Components for cDNA synthesis cocktail

Components	Initial volume (ml)	Initial volume (µl)	Final volume (µl) for 25 Rxns (Vol µl/25)
NO-RT mix	0.1	100	4
RT- super mix	0.1	100	4
Nuclease free water	1.5	150	25
Template (RNA)	-	-	6
Total	-	-	25

Table 2: Cycling conditions for cDNA synthesis

Stage	Temperature (°C)	Time (min)
Primer annealing	25	2
cDNA Synthesis	55	10
Heat Inactivation	95	1

Table 3: Quantitative Polymerase Chain Reaction cocktail reaction set up

Component	Volume per reaction (µl)	Volume per MM (µl)	Final concentration
Mastermix	170	170	90
Forward primer	8.5	5.1	2.7
Reverse primer	8.5	5.1	2.7
Nuclease free water	85	91.8	48.6
Template	4	4	4

Table 4: Contents of primer sequences

Content of primer	Sequence
OREX F	CTTGGCCACCTGAAGACAC
OREX R	GGGTCACCGTAGGCTGAGT
TATA BOX BP F	ATCAAGCCAAGAATTGTTCTGC
TATA BOX BP R	CTTCGTAGATTTCTGCTCGAACT

The Cobb-500 broiler, bred for rapid growth and feed efficiency under stable conditions, showed intermediate expression. This supports observations by (16), who noted that commercially bred chickens exhibit reduced orexin signaling due to the lack of environmental stressors.

Table 5: Fold Change Value for Each Breed Using the Livak Method (2^-ΔΔCT)

Sample	Target	CT Value	HKG	ΔCT	ΔΔCT	2^-ΔΔCT (Fold change)
BT1	**ORX**	31.24	37.78	-6.54	9.78	0.01
BT2	**ORX**	23.46	38.00	-14.54	1.78	0.29
BT3	ORX	21.62	37.94	-16.32	0.00	1.00
BT4	**ORX**	24.95	37.88	-12.93	3.39	0.10
BT5	**ORX**	24.54	37.92	-13.38	2.94	0.13
YE1	**ORX**	28.10	37.78	-9.68	6.64	0.01
YE2	**ORX**	29.02	38.00	-8.98	7.34	0.01
YE3	**ORX**	24.66	37.94	-13.28	3.04	0.12
YE4	**ORX**	26.37	37.88	-11.51	4.81	0.04
YE5	**ORX**	28.14	37.92	-9.78	6.54	0.01
FE1	**ORX**	27.69	37.78	-10.09	6.23	0.01
FE2	**ORX**	22.36	38.00	-15.64	0.68	0.62
FE3	**ORX**	26.68	37.94	-11.26	5.06	0.03
FE4	**ORX**	19.93	37.88	-17.95	-1.63	3.09
FE5	**ORX**	20.80	37.92	-17.12	-0.80	1.75

BT – Exotic Birds, YE – Yoruba Ecotype, FE – Fulani Ecotype

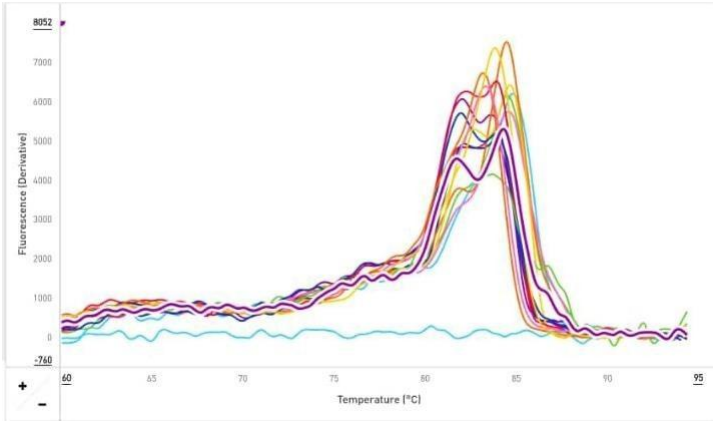

Figure 1: shows individual melt curves, color-coded by breed

Exotic (Cobb-500): Red, orange, yellow, pink, purple, Yoruba ecotype: Light blue, dark blue, light green, dark green, cyan, Fulani ecotype: Light brown, dark brown, violet, magenta, deep pink, The average melt curve values were: Cobb-500: 83.68 FI, Yoruba ecotype: 83.61 FI, Fulani ecotype: 83.61 FI

CONCLUSION AND APPLICATION

Conclusively, Fulani ecotype had the highest expression level, suggesting enhanced physiological adaptability. The Exotic breed showed moderate expression, consistent with its selective breeding for controlled environments while Yoruba ecotype recorded the lowest expression, indicating limited orexin activity. According to the study the Fulani ecotype should be prioritized in breeding and conservation programs. Its elevated orexin expression indicates strong adaptability and energy regulation.

REFERENCES

(1) Ajala, M. K., Nwagu, B. I., and Olayemi, M. E. (2020). The role of poultry farming in food security and economic empowerment in Nigeria. *Nigerian Journal of Animal Production*, 47(3): 52–59.

(2) Adene, D. F., and Oguntade, A. E. (2006). *The structure and importance of the commercial and village-based poultry industry in Nigeria*. Food and Agriculture Organization.

(3) FAO. (2018). *Poultry sector Nigeria: FAO animal production and health livestock country reviews*. No. 11. Rome.

(4) FAO. (2018b). *FAOSTAT statistical database*. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/

(5) Oyeniran, A. O., Balogun, O. S., and Oladimeji, T. T. (2022). Genetic isolation and adaptation of Fulani chickens in Nigeria. *African Journal of Poultry Science*, 14(2): 97–105.

(6) Osaiyuwu, A. I., Odunsi, A. A., and Farinu, G. O. (2011). Assessment of morphological characteristics of Yoruba ecotype chicken. *Nigerian Journal of Animal Production*, 38(1): 78–86.

(7) Ige, A. O., Sola-Ojo, F. E., and Oke, O. E. (2014). Genetic variation in Nigerian indigenous chickens: A tool for conservation and breeding. *International Journal of Poultry Science*, 13(6): 331–338.

(8) Sola-Ojo, F. E., Ayorinde, K. L., and Akinyemi, M. O. (2012). Performance characteristics of Nigerian indigenous chickens in different rearing systems. *International Journal of Agricultural Sciences*, 4(2): 109–114.

(9) Google Earth Map (2024). Geographical location of LAUTECH, Ogbomoso, Oyo State, Nigeria. Google LLC. http://earth.google.com/web/search/lautech+ogbomoso: Date accessed October 25, 2024.

(10) Ufoegbune, G. C., Adedokun, J. A., and Awomeso, J. A. (2008). Evaluation of temperature and humidity conditions in Abeokuta. *Journal of Environmental Sciences*, 12(3): 172–178.

(11) Lasagna, E., Ceccobelli, S., Iannuccelli, N., and Cozzi, M. C. (2020). Genetic conservation and molecular characterization of local chicken breeds. *Animals*, 10(2): 153.

(12) SAS (2003). SAS. Users Guide Statistical Analysis. *Inst. Inc. Cary*. North Caroline.

(13) Mwacharo, J. M., Bjørnstad, G., and Hanotte, O. (2013). Genetic resources and the sustainable production of local chickens: A critical appraisal. *World's Poultry Science Journal*, 69(2): 333–344.

(14) Sakurai, T. (2007). The neural circuit of orexin (hypocretin): Maintaining sleep and wakefulness. *Nature Reviews Neuroscience*, 8(3): 171–181.

(15) Greene, E., Burns, D., and Dridi, S. (2020). Orexin regulates hepatic lipogenesis through leptin signaling in chickens. *Poultry Science*, 99(3): 1403–1412.

(16) Doe, J. A., Lim, B. L., and Zhao, C. (2021). Differential expression of orexin and feeding behavior in broiler chickens under controlled environments. *Poultry Biology Research*, 10(2): 145–152.