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COMPARATIVE EXPRESSION PATTERNS OF LIPOPROTEIN LIPASE AND ASSOCIATED LIPID REGULATING GENES IN DIFFERENT BREEDS OF CHICKENS IN SOUTHERN GUINEA SAVANA OF NIGERIA

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ABSTRACT

Comparative Expression patterns of Lipoprotein Lipase and associated Lipids regulating genes in different breeds of Chickens in Southern Guinea Savana of Nigeria were investigated. A total of 300 day-old birds of mixed sexes were used in this study. One hundred each of the Fulani ecotype, Noiler and Broiler chicken birds were randomly allotted into three experimental treatments, with each treatment replicated into five containing twenty birds per replicate in a completely randomized design (CRD) arrangement, with Fulani ecotype as treatment 1 (T_1), Noilers as treatment 2 (T_2) and Broiler as treatment 3 (T_3 control). The birds were fed an experimental diet containing 21 % crude protein and 2900 Kcal ME/kg in a single-phase feeding regime for 22 weeks for T_1 and T_2 and 8 weeks for T_3. Nine fresh liver samples were collected from each of the three treatments. Lipoprotein lipase (LPL), Apolipoprotein AI (APOA1), Apolipoprotein B (APOB) and Peroxisome proliferator-activated receptor alpha (PPARA) genes were extracted and expressed. Lipoprotein lipase was highly upward regulated in Fulani ecotype chickens with a cycle threshold value of 2.61, while there was a downward regulation of the gene in the Noilers and Broilers with cycle threshold values of -0.97 and -1.64, respectively. Apolipoprotein lipase A-1 (APOA-1) was upwardly regulated in the Broilers

with a cycle threshold value of 0.65. In contrast, this gene was downwardly regulated in the Fulani ecotype and the Noilers, with cycle threshold values of -0.39 and -0.25, respectively. Apolipoprotein B precursor (APOB) showed a highly upward regulation in the Fulani ecotype with a cycle threshold value of 2.19, while there was a downward regulation of the gene in a similar pattern in both the Noilers and Broilers with cycle threshold values -0.1.03 and -1.16, respectively. A slightly upward regulation of Peroxisome proliferator-activated receptor gamma (PPARG) was observed in the Fulani ecotype with a cycle threshold value of 0.23. At the same time, there was a slight downward regulation of the gene in the Noilers and a lesser downward regulation of the gene in the Arbor acre, with cycle threshold values of -0.002 and -2.23, respectively. Understanding the dynamics of this genetic determinant in domestic chicken species in this study will facilitate the development of molecular markers for a prospective marker-assisted selection for improvement of growth and sensory attributes of Indigenous chicken breeds as lipid regulation is known to influence growth and meat sensory attributes.

Keywords: Associated Lipids Regulating Genes, Comparative Gene Expression, Different Breeds of Chickens, Lipoprotein Lipase.

INTRODUCTION

Presently, poultry meat's market structure is changing from producer- to consumer-driven. Poultry producers are encouraged to increase productivity when there is a sustained demand that will guarantee profit maximization. Commercial chicken breeds are characteristically fast growers, high-feed consumers, poor disease resistors, and high-fat accumulators, and they are poor in organoleptic perception by consumers (Debora *et al*., 2017). These properties have always constituted a threat factor and a risk too big for some farmers and investors to undertake. On the other hand, local chicken ecotypes have been reported to have a better organoleptic perception by consumers, a condition thought to be a result of their minimal fat accumulation (Debora *et al*., 2017). Lipoprotein lipase (LPL) has been known to playplay a critical role in regulating the breaking down of fat in triglycerides (Mead *et al.,* 2002a; b). Other genes that exert a regulatory influence on the breaking down of fat in the form of triglycerides are Apolipoprotein AI (APOA1), Apolipoprotein B (APOB), and Peroxisome proliferator-activated receptor alpha (PPARA). Knowledge about these genes has greatly increased over the past decade (Andrade, 2018). Lipoprotein Lipase (LPL) and the associated genes have been reported to control triacylglycerol partitioning between adipose tissue and muscle that increases fat storage or provides energy in fatty acids for muscular growth (Hidayati *et al*., 2015). The

expression pattern of this Lipoprotein lipase and the associated genes in fatty tissues is therefore thought to greatly influence the growth and sensory attributes of different poultry species. So, studying and properly understanding the mechanism of expression of these genes in different poultry species will form a solid foundation for developing molecular markers required to design a probable marker-assisted selection programme to improve growth performance and desired sensory attributes of poultry species in Nigeria. This study was, therefore, conducted to evaluate the expression of lipoprotein lipase (LPL) and associated genes in Fulani ecotype, Noiler and Broiler birds at the lipoprotein lipase and the associated genes loci levels. It is hoped that information from this study will form a foundation for developing genetic markers for a prospective marker-assisted selection to improve indigenous chicken breeds in Nigeria.

MATERIALS AND METHODS

Experimental Location

The research was conducted in the Teaching and Research farm of the Federal University of Technology, Minna, Bosso and Gidan Kwano Campuses, respectively. Laboratory work was conducted in the Department of Animal Production and Biochemistry Laboratories, Federal University of Technology Minna and The African Bioscience Laboratory Ibadan. Bosso Campus is situated between latitude 9o 28′ and 9o 37′ N, longitude 6o 23′ and 6o 33′ E. The Gidan-Kwano campus is situated at latitude 9o 51 ‘N and longitude 6o44 ‘E. The mean annual rainfall of the study area varies from 1102.6mm to 1361.7 mm. The vegetation is Southern Guinea Savannah agro-climatic vegetation. It has an altitude of 147 m above sea level (Njoku *et al*., 2021).

Experimental Materials

The birds used in this study include the Fulani ecotype, Noiler, and Broiler birds. Maize, maize offal, and broiler concentrates were used for diet formulation. Other materials used were feeding and drinking troughs, wood shavings, and wire mesh to construct the pens. Heat sources for brooding birds (electric bulb and charcoal heat source) were also used during the study.

Source of Experimental Materials

Parent stock of the Fulani ecotype fowl was sourced from the open market within Bosso Local Government Area of Niger State, Nigeria, to generate the birds used in this study, while the Noiler and Broiler chicken birds were procured from Amo hatchery, Ibadan. The feed ingredients used in the study (Maize, maize offal, and broiler concentrates) were sourced from

the open markets and an agro mill shop within the Bosso local government area of Niger State. Drugs and vaccines were sourced from an accredited agro-veterinary store within the Minna metropolis.

Experimental Diet and Design

A total of 300 day-old birds of mixed sexes were used in this study. One hundred each of the Fulani ecotype, Noiler and Broiler chicken birds were randomly allotted into three experimental treatments. Each treatment was replicated five times with twenty birds (20) per replicate in a completely randomized design (CRD). The birds were grouped into three treatments with Fulani ecotype as treatment 1 (T_1), Noiler birds as treatment 2 (T_2) and Broiler birds as treatment 3 (T_3 as the control). The birds were fed an experimental diet formulated to contain 21 % crude protein and 2900 Kcal ME/kg (Table 1) in a single-phase feeding regime. Feed and water were served *ad libitum* throughout the experimental duration of 22 weeks for T_1 and T_2, while T_3 was fed for 8 weeks.

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Table 1: Ingredient and Proximate composition of experimental diet

Ingredients	Percentage (%)
Maize	55.32
Maize offal	5.00
Concentrate	39.68
Total weight (kg)	**100.00**
Proximate analysis	
Crude Protein	21.00
Metabolisable Energy (Kcal/kg)	2,900
Moisture content	4.20
Crude fiber	5.50
Crude fat	7.24
Ash	9.00
Nitrogen free extract	53.05

Management of the Experimental Birds

The birds were managed using a deep litter system. Before the arrival of the chicks, the pens were cleaned, disinfected/fumigated, and littered with wood shavings up to 5 cm deep. A charcoal fire maintained the temperature in the brooding house. Daily cleaning, Drug administration, and vaccination were carried out until the birds attained maturity.

Sample Collection and Handling

A total of 9 fresh liver tissue samples were collected from three birds, each of which is a Fulani ecotype, Noiler and Broiler chicken. Five grams of the fresh liver samples were collected using a surgical knife and gently placed into a sterile Eppendorf tube and completely submerged with RNAlater (an aqueous, non-toxic tissue and cell storage reagent that stabilizes and protects cellular RNA intact) solution. The samples were properly labelled and transported in an icepack to African Biosciences Laboratory, JaaGee House, Ibadan-Ife expressway, Ibadan, Oyo state, Nigeria, within 24 hours of collection, where they were kept under -20 ^{o}C in a deep freezer for RNA extraction and Lipoprotein lipase and associated genes Expression studies.

Table 2: Lipoprotein Lipase and the Associated Genes Primer Designs and Description

Gene name	Accession number	Primer sequence	Primer length	Product length	Exon-exon junction
Lipoprotein lipase (LPL), Mrna	NM_205282.2	Forward – GCGACTCAGTTCTACTTCGTG Reverse – TTCATCTCAGCTTCGGGATCG	21 21	250	Yes
Apolipoprotein AI (APOA1), mRNA	NM_205525.5	Forward – TGGGCAAACAGCTTGACCTGA Reverse – CCGTCCACTTGGCAGAGAAC	21 20	216	Yes
Apolipoprotein B (APOB), mRNA	NM_001044633.2	Forward – CTTTAGAGGCCTCCGCCAG Reverse – TGCCTCTCCAGAACCTTTCA	19 20	170	Yes
Peroxisome proliferator activated receptor alpha (PPARA), mRNA	NM_001001464.1	Forward – TAGTAAGCTCTCAGAAACTTTGTTG Reverse – GAAACAGAAGCCGCTTTCCA	25 20	157	Yes

Extraction and Purification of Lipoprotein Lipase Gene/cDNA

The guanidinium thiocyanate-phenol-chloroform method was used to extract genomic mRNA from the liver samples, as Chomczynski and Sacchi (1987) described using the following forward and reverse primers, as shown in Table 2.

Lipoprotein Lipase and Associated Genes Expression/Ct values determination

The extracted Lipoprotein lipase and associated genes mRNA's were converted to their cDNA's using the FIREScript RT cDNA Synthesis KIT according to the procedure explained by Egena *et al.* (2023) and Okolo *et al.* (2023). The process involved using 1ul of Reverse Transcriptase, 2ul of 10x reaction buffer, 0.5ul RNase Inhibitor (Ribogrip), 0.5ul of primers with a 5-uM concentration and 10ul of the RNA sample (at 50ng/µl). Nuclease-free water was used to balance the reaction volume to 20ul. The thermocycling conditions were as follows: Annealing at 25°C for 10 minutes, Reverse Transcription at 45°C for 30 minutes and Enzyme inactivation at 85°C for 5 minutes. The synthesized cDNAs were amplified using the My IQ single-color real-time cycler. TheqPCRmix used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25µl reactions consisting of 4 µl of the 5x HOTFirepolqPCR Mix, 0.4 µl each of the forward and reverse primers and a specific probe, which had a concentration of 250nM, 18.2 µl of Nuclease-free water and 2 µl cDNA template(100ng). The cycling conditions were as follows: Initial Activation at 95°C for 12 minutes, Denaturation at 95°C for 15 seconds, Annealing at 55 and 53°C for 20 seconds (for Lipoprotein lipase and associated genes and GAPDH, respectively and Elongation at 72°C for 20 seconds.

RESULTS AND DISCUSSION

The results of the gene Expression/Cycle threshold value determination Lipoprotein lipase (LPL), Apolipoprotein AI (APOA1), Apolipoprotein B (APOB) and Peroxisome proliferator-activated receptor alpha (PPARA) genes in Different Breeds of Chickens are shown in Figures 1, 2, 3 and 4, respectively. These results are significant as they provide insights into the potential impact of gene expression on fat content in meat. The results in Figure 1 showed a highly upward regulation of the Lipoprotein lipase gene in Fulani ecotype chickens with a cycle threshold value of 2.61, while there was a downward regulation of the gene in both the Noilers and Broiler chickens with cycle threshold values of -0.97 and -1.64, respectively. The high upward regulation of the gene in the Fulani ecotype is indicative of the presence of low-fat content in the meat from this species of chicken. More so, the downward regulation of the gene in Noiler and Broiler birds is suggestive of the presence of high-fat content in these breeds of chicken. However, as seen from the result of the gene expression, the fat content regulates fat concentration and, as such, more fatty meat.

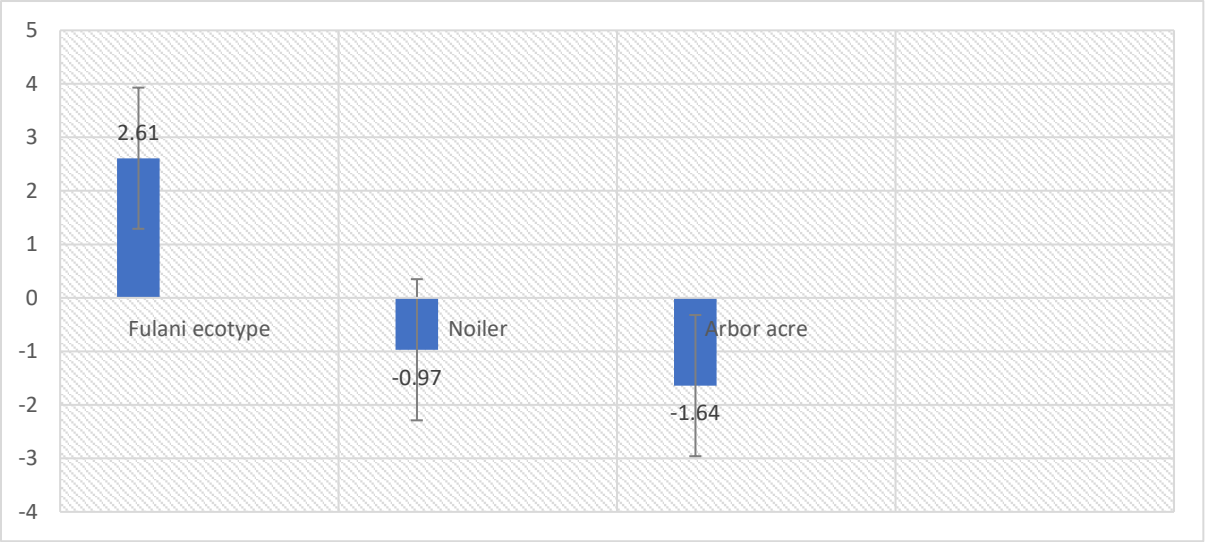

Figure 1: Expression Pattern of Lipoprotein Lipase (LPL) Gene of Three Breeds of Chicken

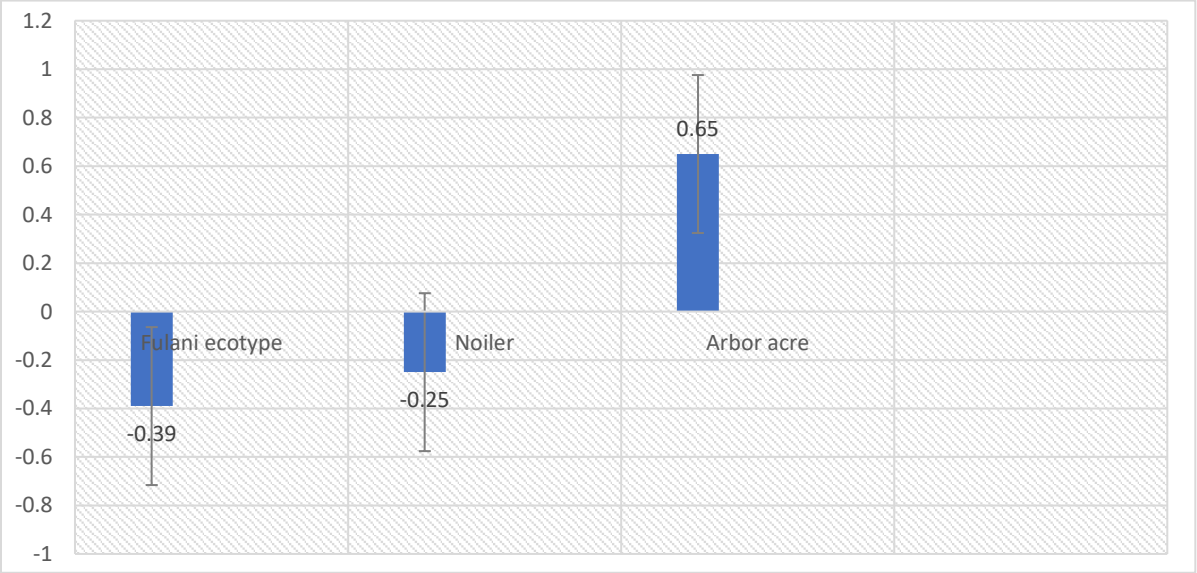

Figure 2: Expression Pattern of Apolipoprotein Lipase A-1 (APOA-1) Gene of Three Breeds of Chicken

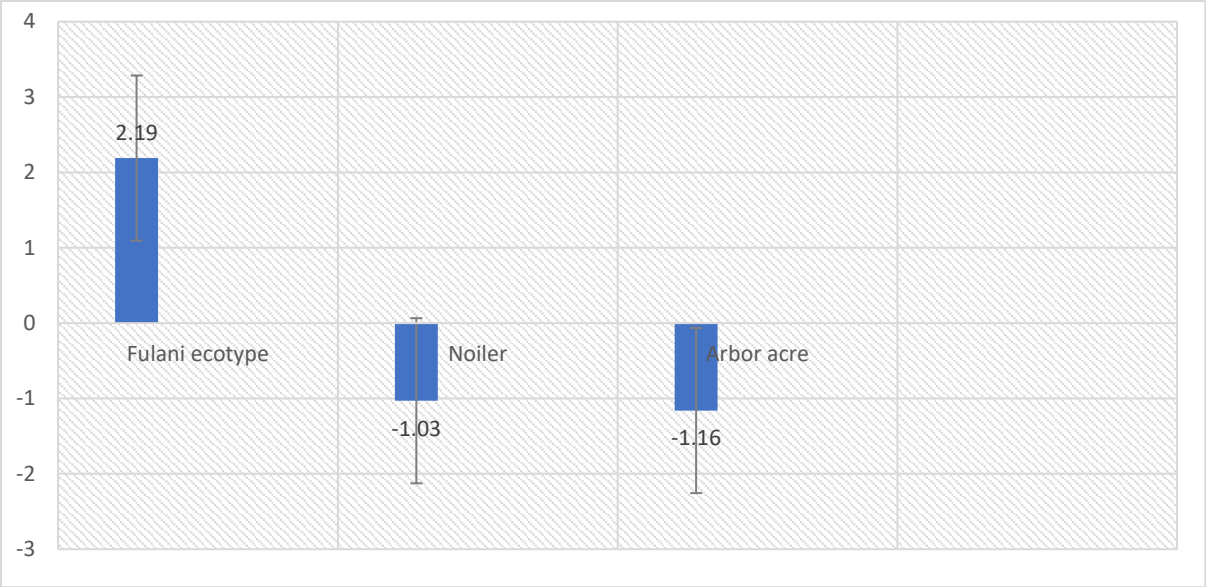

Figure 3: Expression Pattern of Apolipoprotein B Precursor (Apob) Gene of Three Breeds of Chicken

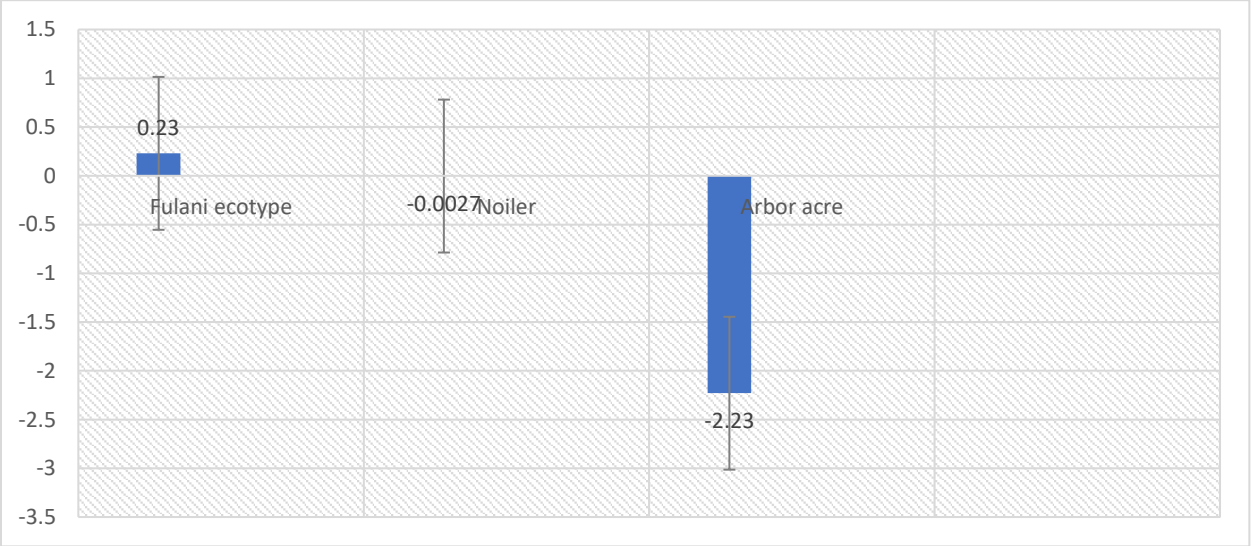

Figure 4: Expression Pattern of Peroxisome Proliferator Activated Receptor Gamma (PPARG) Gene of Three Breeds of Chicken

These results align with the report of Mead *et al.* (2002a; b), who reported that this gene has been known to play a critical role in regulating the breaking down of fat in the form of triglycerides, which could be more in the Arbor acre Broiler chickens than in the Noiler birds as results showed a lower downward regulation of the gene in Broiler chickens than in the

Noiler chickens. The higher the upward regulation, the more the activity of the gene in regulating fat concentration and, as such, more, leaner meat, while the lower the downward regulation, the lesser the activity of The results in Figure 2 showed an upward regulation of Apolipoprotein lipase A-1 (APOA-1) gene in Arbor acre Broiler chickens with a cycle threshold value of 0.65. In contrast, the gene was downwardly regulated in the Fulani ecotype and the Noiler chickens, with cycle threshold values of -0.39 and -0.25, respectively. The high upward regulation of this gene in Arbor acre chicken could be suggestive of high-fat content in the meat from this species of chicken. This result is in line with the description of Jialal and Barton (2016) that the gene is a major protein component of high-density lipids known for regulating cholesterol trafficking and protecting against cardiovascular complications. More so, the downward regulation of the gene in Noiler and Broiler birds suggests the presence of low-fat content in these breeds of chicken. However, as seen from the gene expression results, the fat content could be lower in the Fulani ecotype chickens than in the Noiler birds, as results showed a lower downward regulation of the gene in the Fulani ecotype than in the Noiler chickens. The lower the downward regulation, the more the activity of the gene in regulating fat concentration and, as such, leaner meat, while the lower downward regulation, the lesser the activity of the gene in regulating fat concentration and, as such, more fatty meat.

The results in Figure 3, on the other hand, showed a highly upward regulation of apolipoprotein B precursor (APOB) in Fulani ecotype chickens with a cycle threshold value of 2.19. At the same time, there was a downward regulation of the gene in a similar pattern in the Noilers and Broiler chickens with cycle threshold values -0.1.03 and -1.16, respectively. The high upward regulation of the gene in the Fulani ecotype, as was the case with the Lipoprotein lipase, may also signify the presence of low-fat content in the meat from this species of chicken. More so, the downward gene regulation in Noiler and Broiler birds could measure high-fat content in these chicken breeds. These results agree with Contois *et al.* (2009), who stated that APOB provides a direct measure of the number of atherogenic lipoprotein particles that act as ligands for low-density lipoprotein (LDL) receptor-mediated clearance, resulting in a similar expression pattern as that of the LPL gene. However, as seen from the result of the gene expression, the fat content could be higher in the Arbor Acre Broiler chickens and the Noiler birds, as results showed a lower downward regulation of the gene in Broiler chickens than in the Noiler chickens.

Figure 4 shows a slightly upward regulation of the peroxisome proliferator-activated receptor gamma (PPARG) gene in Fulani ecotype chickens with a cycle threshold value of 0.23. At the same time, there was a slight downward regulation of the gene in the Noilers chickens and a lesser downward regulation of the gene in Arbor acre broiler chickens, with cycle threshhold values of -0.002 and -2.23, respectively. As was the case with the expression of LPL, the results of the PPRAG expression agree with the report of Schoonjans *et al*. (1996) that states that increased activity of LPL may be responsible for the hypotriglyceridemic effects of known activators of various peroxisome proliferators-activated receptors such as PPARG. The up-regulation of the PPARG in the Fulani ecotype indicates low-fat content in this breed's meat. This agrees with the report of Kubota *et al*. (2006) that PPPARG has been known for some time to regulate adipocyte differentiation, fat storage and glucose metabolism. In their separate studies, Sandeep *et al.* (2011) and Kubota *et al*. (2006) also confirmed that activation of PPARG causes insulin sensitization and enhances the expression of several genes encoding proteins involved in glucose and lipid metabolism.

Conclusion

The Expression pattern/Cycle threshold values of Lipoprotein lipase (LPL), Apolipoprotein AI (APOA1), Apolipoprotein B (APOB) and Peroxisome proliferator-activated receptor alpha (PPARA) genes have provided details on the inter-muscular and subcutaneous lipid regulatory capabilities of the different Breeds of Chickens. It is hoped that a better understanding of the dynamics of this genetic determinant in domestic chicken species in this study will facilitate the development of molecular markers for a prospective marker-assisted selection for the improvement of growth and sensory attributes of indigenous chicken breeds in Nigeria.

REFERENCES

Andrade, Jr. M. C. (2018). Lipoprotein Lipase: A General Review. *Insights in Enzyme Research*, 2: 1:3.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, 162(1):156-9. doi: 10.1006/abio.1987.9999. PMID: 2440339.

Contois, J.H., McConnell, J.P., Sethi, A.A., Csako, G., Devaraj, S., Hoefner, D.M. and Warnick, G.R. (2009). AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Apolipoprotein B and cardiovascular disease risk: position

statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. *Clinical Chemistry*, 55(3):407-19.

Debora, C. F. S., Alex, M. V. A. and Alex, A. G. (2017). Quality Characteristics of broiler chickens' meat from free-range and industrial poultry system for consumers. *Journal of Food Science and Technology,* 54(7):1818-1826.

Egena, S. S. A., Otu, B. O., Sikiru, A. B., Adigun, U. O. and Ojimah, C. O. (2023). Interrogating the chicken growth hormone gene of selected Nigerian chicken breeds using DNA-sequencing data. *Journal of Agriculture and Agricultural Technology,* 12 (1): 70-86.

Hidayati, C., Sumantri, R. R., Noor, R., Priyanto, and Rahayu, S. (2015). Single Nucleotide Polymorphisms of Lipoprotein Lipase Gene and its Association with marbling quality in local sheep. *Journal of Indonesian Tropical Animal Agriculture*. 40(1):1-10.

Jialal, I. and Barton, D. P. (2016). Diagnosis of Familial Hypercholesterolemia. *American Journal of Clinical Pathology*, 145(4):437-9. doi: 10.1093/ajcp/aqw001.

Kubota, N., Terauchi, Y., Kumagai, H., Itos, S. and Moroi M. (2006). Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and independent pathways. *Journal of Biological Chemistry*, 281: 8748-8755.

Mead, J. R., Irvine, S. A. and Ramji, D. P. (2002a). The pivotal role of lipoprotein lipase in atherosclerosis. *Cardiovascular Research,* 1; 55(2):261-269.

Mead, J. R., Irvine, S. A. and Ramji, D. P. (2002b). Lipoprotein lipase: structure, function, regulation, and role in disease. *Journal of Molecular Medicine* (Berlin), 80(12):753-769.

Njoku, C. A., Orisakwe, I and Ojeh, V. N. (2021). Mid-afternoon Outdoor Bio-meteorological Conditions in a Hot-humid Climate of Minna in Nigeria, West Africa during the Year 2012. *Journal of Geography, Environment and Earth Science International*, 25(2): 52-62.

Okolo, G. P., Egena, S. S. A., Otu, B. O. and Sikiru, A. B. (2023). Expression of major histocompatibility complex class II gene in commercial strains of broiler chickens administered varying levels of aqueous ginger extract. *Nigerian Journal of Animal Production.* 49 (5):147 – 155. https://doi.org/10.51791/njap.v49i5.3620.

Sandeep, T., Paras, G., Arminder S. S., Chaitnya, K. and Saurabh, S. (2011). The peroxisome proliferator-activated receptor: A family of nuclear receptorsrole in various diseases. *Journal of Advanced Pharmaceutical Technology Resources*, 2 (4): 236-240.

Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.M., Heyma, R. A., Briggs, M. and Deeb, S. (1996). PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the Lipoprotein lipase gene. *Embo Journal,* 15(19): 5336-5348