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Relative Expression of Irf7 Gene in Nigerian Local and Exotic Turkeys and Their Antibody Response to Sheep Red Blood Cell

Oguntade, David Oluwafemi*[1], Orose, Ekinadose[2] and Igene, Fredrick Ugbesia[1]

[1]*Department of Animal and Food Sciences, Ambrose Alli University, Ekpoma, Edo State Nigeria*
[2]*Department of Fisheries Technology, Edo State College of Agriculture and Natural Resources, Iguoriakhi, Edo State Nigeria*

ABSTRACT

The research aimed to determine the expression of the IRF7 gene in the liver and spleen tissues of Nigerian local and exotic turkeys and their immune response to sheep red blood cells. A total of sixty (60) poults, made up of 30 each of Nigerian local and exotic (Hybrid converter) turkeys, were used for this study. It was observed that the antibody titres of Nigerian local turkey were significantly (P< 0.05) higher (0.086) before inoculation, while at day 2, 14 and 21 after inoculation, the exotic turkey had significantly (P< 0.05) higher antibody titres. The CT and relative expression of the IRF7 gene were not significantly (P>0.05) affected by the turkey genotype. The findings demonstrated that exotic turkeys could be successfully raised in a tropical environment as long as sufficient vaccinations were available to promote acquired immunity in this population. Additionally, the data on the IRF7 gene could contribute to the body of knowledge already available on the expression of the IRF7 gene in both local and exotic Nigerian turkeys.

Keywords: *Nigerian local turkey; Exotic turkey; IRF7; Antibody titre; Sheep red blood cell.*

INTRODUCTION

Turkey production in Nigeria has been growing in recent years, as evidenced by an increase in number of turkey farms and turkey meat produced. Turkeys are easy to manage and have a relatively high turnover rate with quick returns on capital investment (1). They are unique among other poultry birds due to their outstanding adaptability to a wide range of environmental conditions and can be successfully reared in different parts of the world, provided they receive adequate nutrition and are properly protected from diseases, predators, and adverse weather (2).

In Nigeria there are two main types of turkey: the exotic and the local turkeys. The major difference between these two turkey populations is productivity and immunity. Exotic turkeys are well-known for higher production potential than local breeds (3). This is because the exotic turkeys have been selected through decades for economic traits such as high body weight, excellent reproductive performance and early maturity.

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Corresponding author: **Oguntade, David Oluwafemi**
E-mail: **oguntadedo@aauekpoma.edu.ng**

The major problem of exotic turkey in the tropics is susceptibility to infectious disease because nearly all the improved breeds of turkey were selected artificially for growth and early maturity in temperate environments. However, their performance under tropical environment needed adequate attention, including intensive medications and high-quality feed for healthy and higher productivity (4). Nigerian local turkeys, on the other hand, are kept mostly under a semi-intensive system (5), well adaptable to adverse climatic conditions of the tropics and survive under low management inputs. According to Ngu *et al*. (6), Nigerian local turkeys are indistinct with multicoloured plumage and in some cases; they appear as pure black, lavender or white. The population of local turkeys in Nigeria is estimated at approximately 1.05 million, making it the least numerous among the major poultry species according to FAOSTAT (7).

It is impossible to overstate the importance of maintaining a healthy flock and reducing medical expenses in order to maximize profits from poultry production. For this reason, the immune system is a perfect subject for multidisciplinary analysis, and hence, turkeys' immune-competence traits must be improved. The immune system plays a crucial role, as it significantly influences productivity and is vital for the survival of any species, particularly in the context of growing concerns about food security. These findings underscore the need to sustain livestock populations that are both highly productive and resilient to diseases.

Traditional approaches involve administering antibiotics, vaccines, and other medications to protect flocks from infections or to manage disease outbreaks. However, Liu *et al.* (8) reported that antibiotics have been causing residues in poultry products and the use of vaccines, if not properly guarded may result in vaccine failure and causes more harms. However, under natural conditions, the response of farm animals to infection is influenced by multiple factors, involving intricate interactions among the host genome, the pathogen, and environmental conditions (9). It is also well established that not all animals exposed to the same pathogens develop disease,

largely due to genetic variation and other contributing factors (9). This suggests that certain animals have an inherent capacity to resist or tolerate infections after exposure or immunization, whereas others are more susceptible.

One of the genes that play key role in immune response of an animal is interferon regulatory factor 7 (IRF7) (10,11). The IRF7 gene codes for a protein with the same name. It regulates all type I interferon (IFN)-mediated immune responses (12) and plays a vital role in protecting against both viral and bacterial infections. In addition, it modulates innate and adaptive immunity while influencing cellular processes such as growth, differentiation, and survival within the host (13, 14). The IRF7 gene, in particular, is recognized as a key regulator of immune function in animals.

Understanding its expression in Nigerian local and exotic turkeys can provide insight into how the immune system of these birds differs from each other. In addition, the knowledge of IRF7 gene expression in the liver and spleen (key immune organs) can help in understanding the role of IRF7 in modulating immunity in turkeys. Therefore, there is need to investigate how the IRF7 gene expressed in Nigerian local and exotic turkeys.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Turkey Breeding Unit of the Teaching and Research Farms, Ambrose Alli University, Ekpoma, while laboratory analyses were carried out at African Biosciences, located at Iyana Agbala, off Iwo Road, Ibadan, Oyo State.

Experimental birds and management

A total of sixty (60) day-old turkeys were used for the study, consisting of thirty (30) Nigerian indigenous birds and thirty (30) Hybrid Converter (exotic) birds. Both breeds were obtained from a reputable hatchery in Ibadan, Oyo State. The birds were brooded for a period of four weeks. At the hatchery, the poults received vaccinations against Marek's disease, Newcastle disease, and infectious bronchitis on the first day. Additional vaccinations for Newcastle disease and fowl pox were administered on day 21 and 48 and repeated at days 42 and 91, respectively. In order to prevent bacterial infection outbreak, antibiotic drugs were administered routinely in accordance with medication guides for turkey production. Both genotypes were reared under an intensive management system using a deep litter housing arrangement. Each bird was individually wing-tagged for proper identification and maintained under uniform management conditions throughout the 16-week experimental period. Feed and clean drinking water were provided ad libitum. The birds were fed commercially formulated turkey diets, beginning with a starter ration from day-old to six (6) weeks of age, followed by a grower diet up to fourteen (14) weeks, and then a finisher diet from week 14 to week 16, which marked the conclusion of the study.

Preparation of Sheep Red Blood Cells (SRBC) and Poults Inoculation

Approximately 5 mL of blood was aseptically collected from a healthy male sheep into an EDTA-containing tube. The sample was centrifuged at 3500 rpm for 5 minutes, after which the supernatant was discarded. The resulting erythrocyte pellet was washed three times with phosphate-buffered saline (PBS), each time centrifuging at 3500 rpm for 5 minutes.

The washed red blood cells were then resuspended in PBS to obtain a 1% SRBC suspension. At six weeks of age, each poult was inoculated with 1 mL of the prepared SRBC suspension via the brachial vein.

Blood and Serum Collection

Approximately 1 mL of blood was collected from each bird through the brachial vein at five weeks of age to establish baseline haematological parameters prior to SRBC administration. Additional blood samples (about 1 mL) were collected on days 14 and 21 post-inoculation to assess the post-primary immune response using haemagglutination assay. Serum samples were separated and stored at −40°C until all analyses were conducted simultaneously. These samples were used to determine total antibody levels and compare antibody titres between the Nigerian indigenous and exotic turkey groups.

Assessment of Antibody Titres by Haemagglutination Assay

Serum obtained from each poult was used to evaluate antibody titres following the haemagglutination procedure described by Wegmann and Smithies (15). The sera were subjected to serial two-fold dilutions in a V-bottom 96-well microtiter plate containing a 1% SRBC suspension. The plates were incubated at 37°C for 45 minutes before reading. Antibody titres were expressed as the base-2 logarithm ($\log_2$) of the reciprocal of the highest dilution that exhibited complete haemagglutination.

Expression analysis of Interferon regulatory factor 7 (IRF 7) gene

Published primers for IRF7 and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes in turkey were used (Table 1) (16). The GAPDH gene was used as housekeeping gene for normalization of IRF7 mRNA.

Table 1: The primers sequences for expression analysis of IRF7 and GAPDH genes in Turkey (Meleagris gallopavo)

Gene	Primer sequence (5VY3V)	Orientation	Authours
IRF-7 gene in turkey	TACACTGAGGACTTGCTGGAGGAGGT	Forward	Wang *et al.* (16)
	AAGATGGTGGTCTCCTGATCC	Reverse	
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	ACGACCAGGTTGTCTCCTGT	Forward	Wang *et al.* (16)
	CCATCAAGTCCACAACACGG	Reverse	

Six turkeys were randomly selected from each of Nigerian local and exotic turkeys, at sixteen weeks of age to extract the total RNA in the liver and spleen tissues. About 1 g of tissue samples were collected and stored in a collection tube under RNA later/shield until used. Tissue samples of liver and spleen tissues obtained from the exotic birds and the three Nigerian local turkey genotypes were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) using a mortar and micro-pestle. Total RNA was subsequently isolated with an RNA extraction kit following the manufacturer's instructions (Invitrogen, Carlsbad, CA).

RNA concentration was quantified by measuring absorbance at 260 nm using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware). The integrity and purity of the RNA were verified by ensuring an A260/A280 ratio within the range of 1.8–2.0. Subsequently, 500 ng of total RNA was reverse-transcribed into first-strand complementary DNA (cDNA) using the PrimeScript RT Reagent Kit (TaKaRa, Dalian, China), following the manufacturer's guidelines.

The resulting cDNA was amplified with a MyiQ single-colour real-time PCR system. Amplification was performed using Solis Biodyne 5× HOT FIREPol qPCR Supermix Plus in a total reaction volume of 25 µL, comprising 4 µL of the 5× qPCR mix, 0.4 µL each of forward and reverse primers along with a specific probe (250 nM), 18.2 µL of nuclease-free water, and 2 µL of cDNA template (100 ng).

Thermal cycling conditions included an initial activation step at 95°C for 12 minutes, followed by denaturation at 95°C for 15 seconds, annealing at 55°C, 56°C, and 53°C (for IRF-7 and GAPDH, respectively) for 20 seconds, and extension at 72°C for 20 seconds. After amplification, melting curve analysis and agarose gel electrophoresis were carried out to confirm primer specificity and the presence of a single amplification product.

Finally, normalized average cycle threshold (Ct) values were used to determine the relative expression levels of the target gene using the 2^−ΔΔCt method (17).

Data Analysis

Data on antibody titres, cycle threshold (Ct) values, and relative expression (R) of the IRF-7 gene in liver tissues of both exotic and Nigerian local turkeys were analyzed using analysis of variance (ANOVA) under the General Linear Model procedure of SAS software (SAS Institute Inc.). Differences among means were determined using Duncan's multiple range test within the same statistical package. The antibody titres data were transformed using logarithmic transformation (log_2) before subjected to analysis of variance.

RESULTS

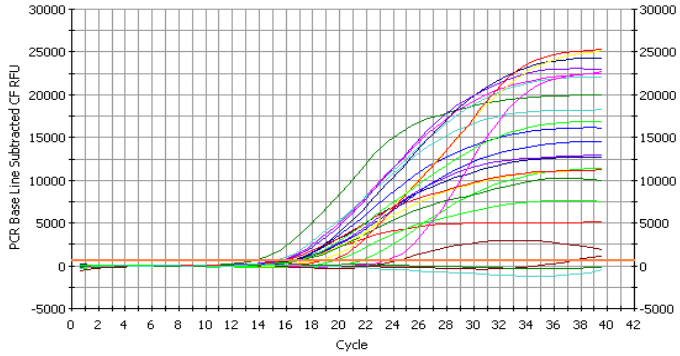

Figure 1: PCR Amplification/Cycle Graph for GAPDH

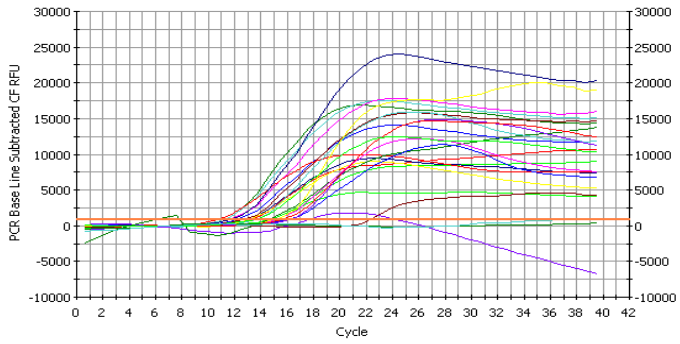

Figure 2: PCR Amplification/Cycle Graph for IRF7

Antibody titres of exotic and Nigerian local turkeys challenged with sheep red blood cells

Table 2 shows the antibody titres of exotic and Nigerian local turkeys before and after inoculation with sheep red blood cell. The results indicate that turkey genotype had a significant effect (P < 0.05) on antibody titres in both Nigerian local and exotic turkeys, both prior to and following inoculation with sheep red blood cells. Before inoculation, the Nigerian local turkeys exhibited significantly higher antibody titres (0.086) compared to the exotic turkeys (0.067, P < 0.05). However, by day 2 post-inoculation, the trend reversed, with the exotic turkeys showing significantly higher antibody titres (0.129) than the Nigerian local turkeys (0.074, P < 0.05). Conversely, at day 7 post inoculation with sheep red blood cell, the Nigerian local turkey had a higher (P<0.05) antibody titres (0.216) than exotic turkey (0.171).

However at Day 14 after inoculation, the exotic turkey had a significantly (P< 0.05) higher antibody titre (0.2121) than Nigerian local turkey (0.178). Similarly, at day 21 after inoculation the exotic turkeys had a higher (P< 0.05) value of antibody titre (0.1489) than Nigerian local turkey (0.0369).

Table 2: Antibody titres of Exotic and Nigerian local Turkeys before and after challenged with sheep red blood cell

Sampling time/Genotype	Nigerian local turkey	Exotic turkey
Before inoculation with SRBC	0.086 ± 0.023^{a}	0.067±0.017^{b}
Day 2 after inoculation with SRBC	0.074±0.020^{b}	0.129 ± 0.046^{a}
Day 7 after inoculation with SRBC	0.216±0.031^{a}	0.171±0.045^{b}
Day 14 after inoculation with SRBC	0.178±0.461^{b}	0.2121±0.6613^{a}
Day 21 after inoculation with SRBC	0.0369±0.0092^{b}	0.1489±0.0412^{a}

Means in the same row with different superscripts a and b are significantly (P<0.05) different

Change in circle threshold (△CT) and relative expression (R) of Interferon regulatory factor 7 (IRF7) in the liver and spleen tissues of Nigerian local and exotic turkeys.

The results of analysis of △CT and relative expression (R) of IRF7 gene in the liver and spleen tissue of Nigerian local and exotic turkeys inoculated with sheep red blood cells (SRBC) were shown in Table 3. The △CT values were negative for both turkey genotypes. Furthermore, neither △CT nor the relative expression of the IRF7 gene showed a significant effect (P > 0.05) based on genotype. However, in the liver tissue, the Nigerian local turkey had a higher value of R (1.93) than the exotic turkey (1.52) while the △CT was higher in exotic turkey (-3.84) than in Nigerian local turkey (-5.21).

On the other hand, Nigerian local turkey had a higher ΔCT but a lower Relative expression (1.37) as compared to exotic turkey which had a lower ΔCT (-8.00) and a higher Relative expression (2.77) of IRF7 gene in the spleen tissue.

Table 3: Change in cycle threshold (CT) and relative expression (R) of IRF7 in the liver and spleen tissues of Nigerian local and exotic turkeys

Tissues	Genotype	△CT	Relative expression
Liver	Nigerian local turkey	-5.21±1.05	1.93±0.31^{NS}
	Exotic turkey	-3.84±1.50	1.52± 0.45^{NS}
Spleen	Nigerian local turkey	-8.00 ± 4.13	2.77 ± 1.24^{NS}
	Exotic turkey	-3.36 ± 1.24	1.37 ± 0.37^{NS}

NS means not significantly (P>0.05) different

DISCUSSION

Antibody titres of exotic and Nigerian local turkeys before and after challenged with red blood cell

In this current study the antibody titres of turkeys were significantly influenced by the genotypes. The Nigerian local turkeys exhibited significantly higher antibody titres prior to inoculation with sheep red blood cells. This outcome is consistent with expectations, as these birds have been primarily selected for adaptation to tropical conditions (2, 19) that are characterize with high rate of infectious disease. This implies a higher capacity of Nigerian local turkey to develop innate immunity as compare to exotic turkey.

However, the higher antibody titres recorded among exotic turkey at 2nd, 14th and 21st days post inoculation imply that the exotic turkeys are sero-protected from the 14th day of

inoculation. It further suggests that exotic turkeys have capacity to develop acquired immunity whenever there is exposure to infectious pathogen. This collaborate the report according to Brito *et al*. (20) that rapid antibody response can play a crucial role in defending against infectious agents, as it is triggered upon exposure to a pathogen. This response may also indicate the development of long-lasting immunological memory, which is a key characteristic of the immune system's ability to remember and respond more effectively to previous infections (21). This suggests that the immunity of exotic in the tropics is highly susceptible to exposure to the disease causing organism. The existence of significant differences in antibody titres in the two breeds of turkeys is therefore an indication that they can be ranked differently in response to a challenge from infectious organisms.

Change in circle threshold (△CT) and relative expression (R) of interferon regulatory factor 7 gene in the liver and spleen tissues of Nigerian local and exotic turkeys

Interferon regulatory factor 7 (IRF7) serves as a key regulator of type I interferon gene expression during infection (12) and is also involved in the differentiation of monocytes into macrophages (22). The findings of this study indicate that the IRF7 gene is expressed in the liver and spleen tissues of both Nigerian local and exotic turkeys. These observations are consistent with previous reports of Irving *et al*. (23) who observed the expression of IRF7 gene at a higher rate in the liver tissue of birds. Chang *et al*. (24) also reported high level of IRF7 expression in the liver, small intestine and large intestine of 60 day old dog.

The IRF7 gene showed higher expression levels in Nigerian local turkeys compared to exotic turkeys in both liver and spleen tissues, even though they are not significantly different. This suggests that IRF7 gene might differ in degree in the roles it plays in both turkey populations. According to Qing and Liu (25), IRF7 gene plays an important role in innate ability of an animal to defend the body against DNA and RNA viruses. This further implies that Nigerian local turkey might be more stable and not affected by the antigen from sheep red blood cell. Nevertheless, the splenic and hepatic expression of IRF7 gene in both turkey populations agreed with the report of (26) that IRF7 gene plays a role in cells type specific manner in the context of infection. Moreover, the non-significant difference observed in the relative expression of IRF7 gene in the liver and spleen tissues suggests that it plays similar roles in both turkey populations. In addition, the expression of the IRF7 gene in sheep red blood cell-challenged turkeys collaborate the postulation that IRF7 play an important role in the early control of blood-borne pathogens (27). This implies that IRF7 gene may plays important role in regulating non- viral infections (27).

CONCLUSIONS

This study demonstrated that turkey genotype affected antibody titres in both Nigerian local and exotic turkeys, both prior to and following inoculation with sheep red blood cells. The Nigerian local turkeys had the higher antibody titres before inoculation while the exotic turkeys had the higher antibody titres after inoculation with sheep red blood cell. The IRF7 gene expressed in both Nigerian local and exotic turkeys; however the expression of IRF7 gene was not significantly influenced by the turkey genotype. This study, therefore suggests that the exotic turkeys could be raised successfully in the tropical environment provided adequate vaccinations are available to stimulate acquired immunity in this population.

More so, the data obtained on IRF7 gene for both turkey genotypes could serve as additional information to the previous literature on IRF7 gene expression in Nigerian local and exotic turkeys.

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