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Characteristics of Intron 17 and Exon 18 of Leptin Receptor Gene in Nigerian Locally Adapted and Exotic Turkeys

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Abstract

Leptin Receptor gene (LEPR) gene has been implicated as the activator of the JAK-STAT signaling pathway and plays an important role in regulating body energy storage and metabolism. Identification of Single nucleotide polymorphisms (SNPs) were carried out in intron 17 and exon 18 of LEPR gene in 200 turkeys comprising 100 each of Nigerian locally adapted and exotic (Nicholas White) turkeys. Blood samples for DNA extraction were collected at 20 weeks. DNA extraction was carried out using whole blood collected with Quick- DNA Miniprep Plus extraction kits by strictly followed the manufacturer's protocol. The sequence data obtained were analyzed using GeneScan and Sequencing software (Applied Biosystems). The single nucleotide polymorphisms (SNPs) present in LEPR gene of the two turkey genotypes were identified using DnaSP 6 software. The allele frequency of each SNP, Heterozygosity (He) and Polymorphic information content (PIC) of the SNPs were calculated. Out of six SNPs identified in intron 17 of the turkey LEPR gene, four were found in local turkeys while all the six were found in exotic turkey. The major allele frequency of the SNPs ranged from 0.86 to 0.96. The SNP 28C>G in exotic turkey had the highest HE while SNPs 7T>G, 67A>T and 85G>C in local turkey had the least. The PIC ranged from 0.077 in local turkey to 0.211 in exotic turkey. In addition, ten SNPs were identified in exon 18 of the turkey LEPR gene, out of which eight were detected in local turkeys while seven were detected in exotic turkeys. The major allele frequency of the SNPs identified in exon 18 ranged from 0.54 in 602A>C in local turkey to 1.00 in 60T>G, 267A>G, 274A>G alleles that are fixed in both local and exotic turkeys. Also, SNP 602A>C in local turkey had the highest HE while incidentally SNPs 130G>C, 166G>A and 789G>A also in local turkey had the least heterozygosity. PIC discovered in exon 18 of LEPR gene ranged from 0.077 to 0.377 in local turkey. Intron 17 and exon 18 of LEPR gene is polymorphic in both Nigerian locally adapted and exotic turkeys and therefore could be used for further analysis to form a basis for genetic variation in growth in the two genotypes.

Keywords: Leptin Receptor Gene; Single Nucleotide Polymorphism; Polymorphic Information Cite; Allele Frequency; Exotic Turkey; Nigerian Locally Adapted Turkey

Introduction

Leptin (from the Greek leptos, meaning thin) is a 16-kilo dalton adipose derived hormone, which functions in energy intake and expenditure, appetite control, metabolism, behaviour and reproductive function [1]. The hormone leptin has an amino-terminal secretory signal sequence of 21 amino acids and is translated as a 167 amino acid protein. This functional signal sequence, results in the translocation of leptin into microsomes, with the subsequent removal of signal peptide [1]. Leptin is one of the key players in regulation of body weight homeostasis. The primary mechanism and function of leptin in the body is not ambiguous. The main role of this hormone is to signal energy sufficiency status to the brain thereby activates specific neural and neuroendocrine pathways [2] that modulate food intake and energy expenditure in an attempt to maintain energy stores and body weight at a set level [3]. Leptin is also involved in reproductive processes and is recognized as a hormonal factor that links adiposity with reproduction in mammals. In addition, leptin treatment accelerates the onset of puberty and restores the effects of delay caused by food restriction [4,5]. These pleiotropic effects of leptin on different organ/systems including various regulations and physiological functions depend on signaling through the leptin receptor (LEPR) gene. Leptin has been shown to act as a satiety signal via hypothalamic LEPR in mammals [6]. LEPR activates the JAK-STAT signaling pathway and plays an important role in regulating body energy storage and metabolism [7,8]. Many studies have been conducted on leptin receptor gene in mammalian species including Mice [9], sheep [10], swine [11], human [12] and cattle [13] but there are scantly of information on the role of LEPR gene in tropical and temperate turkey breeds.

Nigeria indigenous turkeys are hardy and possessed stronger immunity against many infectious diseases of turkey in the tropics. They are characterized and identified with different plumage colours and they include pure black or white or lavender [14]. The productivity of Nigerian indigenous turkeys is relatively low as compared with their exotic counterparts. However, the Nigerian indigenous turkeys can be improved through systematic crossbreeding of exotic Toms with superior heritable traits with indigenous hens. The exotic turkeys are noted for their body weight and early maturity while the Nigeria indigenous turkeys are advantageous for their adaptive nature to the climatic conditions of the country and their disease resistance and environmental tolerance abilities [15].

In humans, LEPR mutations have been reported and associated with obesity and type 2 diabetes [16,17]. Similarly, the rs9436746 polymorphism was found in a LEPR intron and associated with excess weight in humans [18]. In cattle, LEPR mRNA abundance was increased by acute feed restriction [19]. In addition, knockdown of LEPR in neurons of the medial nucleus *tractus solitarius* and the area postrema of rats resulted in significant hyperphagia [20]. Identification of polymorphism(s) in LEPR gene in turkey breeds would be useful as preliminary study in investigating genetic variation in feed intake, feed efficiency ratio, growth and reproductive performances and offer a basis for genetic improvement of tropical breeds of turkeys.

Materials and Methods

Description of Experimental Site and Birds

This research work was carried out at the Turkey Breeding unit of the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Ambrose Alli University, Ekpoma Edo state, Nigeria. The farm lies between 6.44°N and 6.8°E in Esan West Local Government Area of Edo State. It experiences tropical climate with about 1556mm as its mean annual rainfall. The vegetation represents a boundary between the tropical rainforest and the derived savannah. Temperature ranges from 21°C in December to 34°C in February. Relative humidity ranges from 61% in January to 92% in August with yearly average of 82%.

A total of two hundred (200) turkeys comprising of one hundred (100) indigenous/local (Nigerian locally adapted) and one hundred (100) Nicholas white (exotic) day old poults was used for this study. The two breeds were sourced from a reputable hatchery in Ibadan, Oyo state Nigeria. The poults were brooded for four weeks, during which adequate heat, ventilation, medication and feeding were provided. The poults were vaccinated against Marek's disease, Newcastle disease and infectious bronchitis at day old from the hatchery.

Other medications including prophylactic antibiotic drugs were given as required. All the experimental birds were wing tagged for proper identification and subjected to the same management practices throughout the experimental period of 20 weeks. Water and commercial feed were provided *ad libitum*. They were fed starter ration of about 28 percent crude protein and 2860 Kcal Metabolisable energy/kg from day old to six (6) weeks old. This was followed by a 24% crude protein and 2900Kcal Metabolisable energy/kg of finisher mash till the end of the experiment

DNA Extraction

Blood samples for DNA extraction were collected at 20 weeks. A milliliter of blood was collected through the brachial vein using sterile needle and syringe into ethylene diamine tetra acetic acid (EDTA) bottle, to prevent coagulation. DNA extraction was carried out using whole

blood collected with Zymo research quick-gDNA™ miniprep kit. The protocol includes; to each 20mg tube of proteinase K, 1060µl of storage buffer was added and stored at -20°C. Then, up to 200µl of whole blood of turkey was taken into microcentrifuge tube and 200µl Biofluid and cell buffer and 20µl proteinase K were added. The mixture was then mixed thoroughly before incubated the tube at 55°C for 10 minutes. Thereafter, 1volume Genomic binding buffer was added to the digested sample and then mixed thoroughly. The mixture was then transferred to a Zymo-spin IIC-XL column in a collection tube and centrifuged (≥12,000xg) for 1 minutes. The collection tube with the flow through was discarded. Then, 400µl DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for 1 minute. The collection tube was emptied and 700µl g-DNA wash buffer was added and centrifuged for 1 minute. The collection tube was again emptied and 200µl g-DNA wash buffer was added and centrifuged for 1 minute. At this time, the collection tube with the flow through was discarded. To elude the DNA, the mixture was transferred to a clean microcentrifuge tube and more than 50µl DNA elusion buffer was added and incubated for 5 minutes, and then centrifuged for 1 minute.

Primer Design and Amplification of DNA

A pair of primers was designed to amplify intron 17 and exon 18 of Leptin receptor gene in turkey which is 950bp in length using NCBI primer blast (Ensembl release 104 - May 2021 © EMBL-EBI). The amplification reaction was carried out in a micro centrifuge tube using programmable thermocycler (Mastercycler pro by Eppendorf). PCR amplification was performed in a volume of 25µL mixture containing 2µl of DNA template, 1µl of each forward and reverse primer, and 12µL of master mix and 9µl of sterilized distilled water. Electrophoresis was carried out on 2µl sample from the PCR amplicon at 100 volts 45 mins in 1% agarose gel in 1x TAE buffer containing 1.0µl strain of ethidium bromide. After electrophoresis, the gel was taken into gel documentation machine to view the bands in the gel by Ultra – violet illumination.

Sequencing of Amplified DNA

A total of 25µl comprising 20 ng of purified DNA and 25 µl of PCR mix (1 µl of 5mMdNTP, 1 µl of 10Mm of each primer, 2.5 µl of 25mM of $MgCl_2$, 2.5 µl of 10x PCR buffer, 16.8 µl of Nuclease free H_2O and 50.2 µl of 10U/ µL of Surf Hot Taq DNA) (Stabvida, Spain) was used for direct sequencing of intron 17 and exon 18 of LEPR gene on a BDTv3.1 (Applied Biosystems, USA) technology. The reaction mixtures were then transferred to a 96-well reaction plates of ABI 3730 XL Capillary DNA Analyzer (Applied Biosystems, USA), and the cycling parameters indludes: 96°C for 1 minute for initial denaturation, followed by 30 cycles at 96°C for 10 seconds of denaturation, annealing at 50°C for 6 seconds, 60°C for 4 minutes of extension, and final extension step at 12°C for 10 minutes. The sequence data obtained were analyzed using GeneScan and Sequencing software (Applied Biosystems).

Sequence Analysis

After sequencing, the nucleotide sequences were cleaned and edited using BIOEDIT and MEGA 6 software to remove noises in the sequences. Further, Bioedit and MEGA 6 softwares [21] were also used to align the sequences obtained. The pairwise and multiple alignments of the sequences were done using ClustalW software [22] incorporated in MEGA 6 software [21]. The aligned sequences were saved in FASTA format for singe nucleotide polymorphism (SNP) detection.

Identification and analysis of single nucleotide polymorphism

The SNP present in LEPR gene of the two turkey genotypes were identified using DnaSP 6 software [23]. The allele frequency of each SNP identified was determined by dividing the frequency of each allele with the total sample size for each genotype.

Heterozygosity (He) of the SNPs was calculated using the formula proposed by [24] as shown below:

$$He = 1 - (p^2 + q^2)$$

Where 'p' is the major allele frequency and 'q' is the minor allele frequency.

The Polymorphic information content (PIC) of the SNPs was calculated using the formula proposed by [25] as shown below:

$$PIC = He - 2p^2q^2$$

Where 'p' is the major allele frequency and 'q' is the minor allele frequency.

Results

Polymorphisms Identified in Intron 17 of Leptin Gene in Local and Exotic Turkeys

The 214 bp intronic region before exon 18 remained after cleaning was used for the analysis. Single nucleotide polymorphisms (SNPs) identified in intron 17 of LEPR gene in local and exotic turkeys are presented in Table 1. A total of six SNPs were detected in the turkeys. Only four polymorphisms were detected in local turkeys while all the six were detected in exotic turkey. The four SNPs detected in local turkeys

(7T>G, 28C>G, 67A>T, 85G>C) were shared with the exotic turkey while the other two were only detected in the exotic turkey (17T>G, 134T>G). Five of the SNPs identified are singleton while only one (28C>G) is parsimonious.

Region	SNP	Genotype where SNP occurs	Reference allele	Type of polymorphism
Intron19	7T>G	Local, Exotic	T	Singleton
	17T>G	Exotic	T	Singleton
	28C>G	Local, Exotic	C	Parsimony
	67A>T	Local, Exotic	A	Singleton
	85G>C	Local, Exotic	G	Singleton
	134T>G	Exotic	T	Singleton

Note: Polymorphism nomenclature is based on its position in the intron.
Table 1: Polymorphisms Identified in intron 17 of LEPR Gene in Local and Exotic Turkeys.

Major Allele Frequencies (MAF), Heterozygosity and Polymorphic Information Content of SNPs identified in intron 17 of LEPR Gene in Local and Exotic Turkeys

The allele frequency, heterozygosity and polymorphic information content of SNPs identified in intron 17 of LEPR gene of local and exotic turkeys are presented in Table 2. The major allele frequency of SNPs identified in LEPR gene of intron 17 ranged from 0.86 to 0.96 with mutation 28C>G in exotic turkey having the least MAF.

The heterozygosity of the SNP observed in the intron 17 of turkey LEPR gene ranged from 0.08 to 0.24. SNP 28C>G in exotic turkey had the highest heterozygosity while incidentally SNP 7T>G, 67A>T and 85G>C all in local turkey had the least heterozygosity.

Polymorphic information content (PIC) observed in intron 17 of LEPR gene ranged from 0.077 all in local turkey polymorphisms to 0.211 in 28C>G polymorphism in exotic turkey.

Region	Genotype	SNP	Allele frequency	Major Allele	Heterozygosity	PIC
Intron 17	Local	7T>G	0.04	0.96	0.08	0.077
	Exotic	7T>G	0.05	0.95	0.1	0.095
	Exotic	17T>G	0.05	0.95	0.1	0.095
	Local	28C>G	0.12	0.88	0.21	0.188
	Exotic	28C>G	0.14	0.86	0.24	0.211
	Local	67A>T	0.04	0.96	0.08	0.077
	Exotic	67A>T	0.09	0.91	0.16	0.147
	Local	85G>C	0.04	0.96	0.08	0.077
	Exotic	85G>C	0.05	0.95	0.1	0.095
	Exotic	134T>G	0.05	0.95	0.1	0.095

Table 2: Major Allele Frequencies, Heterozygosity and Polymorphic Information Content of SNPs Identified in intron 17 of LEPR gene of Local and Exotic Turkeys.

Polymorphisms Identified in Exon 18 of LEPR Gene in Local and Exotic Turkeys

The 844 bp region of exon 18 remained after cleaning was used for the analysis. Single nucleotide polymorphisms (SNPs) identified in exon 18 of LEPR gene in local and exotic turkeys are presented in Table 3. A total of ten single nucleotide polymorphisms (SNPs) were detected. Eight SNPs were detected in local turkeys while seven were detected in exotic turkey. Five of the SNPs were shared between the two populations (60T>G, 130G>C, 267A>G, 274A>G, 602A>C) while three (166G>A, 789G>A, 822T>A) were specific to local turkey population and two (40G>C, 99T>G) specific to the exotic turkey population.

Region	SNP	Genotype where SNP occurs	Reference allele	Type of polymorphism
Exon 18	40G>C	Exotic	G	Singleton
	60T>G	Local, Exotic	T	Fixed
	99T>G	Exotic	T	Singleton
	130G>C	Local, Exotic	G	Singleton
	166G>A	Local	G	Singleton
	267A>G	Local, Exotic	A	Fixed
	274A>G	Local, Exotic	A	Fixed
	602A>C	Local, Exotic	A	Parsimony
	789G>A	Local	A	Singleton
	822T>A	Local	T	Parsimony

Note: Polymorphism nomenclature is based on its position in the exon

Table 3: Polymorphisms Identified in exon 18 of LEPR Gene in Local and Exotic Turkeys.

Major Allele Frequencies, Heterozygosity and Polymorphic Information Content of SNPs Identified in Exon 18 of LEPR Gene in Local and Exotic Turkeys

The allele frequency, heterozygosity and polymorphic information content of SNPs identified in exon 18 of LEPR gene of local and exotic turkeys are presented in Table 4. The major allele frequency of the SNPs identified in exon 18 of turkey LEPR gene ranged from 0.54 in 602A>C polymorphism in local turkey to 1.00 in 60T>G, 267A>G, 274A>G alleles that are fixed in both local and exotic turkeys.

The heterozygosity of the SNPs observed in the exon 18 of LEPR gene ranged from 0.08 to 0.50. SNP 602A>C in local turkey had the highest heterozygosity while incidentally SNP 130G>C, 166G>A and 789G>A also in local turkey had the least heterozygosity.

Polymorphic information content (PIC) discovered in exon 18 of LEPR gene ranged from 0.077 to 0.377in 602A>C all in local turkey.

Region	Genotype	SNP	Allele frequency	Major Allele frequency	Heterozygosity	PIC
Exon 18	Exotic	40G>C	0.05	0.95	0.1	0.095
	Local, Exotic	60T>G	-	1	-	
	Exotic	99T>G	0.05	0.95	0.1	0.095
	Local	130G>C	0.04	0.96	0.08	0.077
	Exotic	130G>C	0.05	0.95	0.1	0.095
	Local	166G>A	0.04	0.96	0.08	0.077
	Local, Exotic	267A>G	-	1	-	
	Local, Exotic	274A>G	-	1	-	
	Local	602A>C	0.46	0.54	0.5	0.377
	Exotic	602A>C	0.27	0.73	0.39	0.312
	Local	789G>A	0.04	0.96	0.08	0.077
	Local	829T>A	0.08	0.92	0.15	0.139

Table 4: Major Allele Frequencies, Heterozygosity and Polymorphic Information Content of SNPs Identified in exon 18 of LEPR gene of Local and Exotic Turkeys.

Discussion

Genetic variability within and among populations is an important tool for domestic animal improvement and species adaptability. The effectiveness of selection and crossbreeding depend on the amount of genetic variation. The presence of polymorphisms in intron 17 and exon 18 of LEPR gene in local and exotic turkeys was an indication that

these regions are polymorphic and could be exploited for the development of improved locally adapted turkey in Nigeria.

Allele frequency describes the proportion of gene copies that are of a particular allele in a defined population and ranges from 0 when there is an absence of the particular variant in the population to 1 when the variant type observed is the only allele present [26,27] . Where the allele frequency is 1, the population is said to be fixed for that particular allele and therefore not segregating [28]. Allele frequency are generally low which signify occurrence of more singletons than parsimony mutations in the intron in local turkey while it ranges from low to medium in exotic turkey. The observation in exotic turkey is not surprising as the breed has been highly selected for meat production which would have fixed majority of important alleles. Three alleles are fixed in the two populations and different from the ones in the reference turkey sequence which may be important to local adaptation of these turkeys to tropical environment.

Heterozygosity quantifies within individual genetic diversity and is also related to inbreeding. Low heterozygosity can affect fitness in natural settings and is always common in highly selected population as in the case of exotic turkey that has been largely selected for optimum meat production [29-31]. The Polymorphic Information Content (PIC) value is usually used in measuring the informativeness of a genetic marker for linkage studies [24]. The PIC in this study ranged from low to medium in exotic intron sequence while it is generally low in local turkey. The PIC for the exon 18 of both local and exotic turkey ranged from 0.077and 0.095 to 0.312 and 0.377 respectively. This is in agreement with Hildebrand, et al. [32] who reported that maximum PIC value for SNPs that are bi-allelic markers is 0.375. This implies that the markers are polymorphic as well as informative for linkage analysis. Therefore, any local and exotic turkey chosen at random at these loci will likely be heterozygous for these markers.

Conclusion

Analysis of intron 17 and exon 18 of LEPR gene in Nigerian locally adapted and exotic (Nicholas White) turkeys showed that the region is polymorphic in the two breeds. However, most of the SNPs identified are singleton while few are fixed and others parsimonious. This study provides basis for further study on the role of LEPR gene in turkey breeds especially as related to genetic variation in feed intake, body weight and other production traits.

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