DIFFERENTIAL EXPRESSION AND CYCLE THRESHOLD OF MYOSTATIN IN NIGERIAN LOCALLY ADAPTED AND EXOTIC TURKEYS

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ABSTRACT
Myostatin (MSTN) gene plays a key role in the regulation of myogenesis. This study focused on the relative and differential expression of MSTN gene in Nigerian locally adapted and exotic turkeys. A total of two hundred turkeys comprising one hundred and fifty Nigerian locally adapted (fifty each of black, lavender and white colour variants) and fifty Hybrid converter (exotic) day-old poults were used for this study. The total RNA in liver, breast and thigh muscle tissues was extracted at six weeks (grower stage) and sixteen weeks (finisher stage) of age. The cycle threshold (CT), delta CT and relative expression (R) values of MSTN gene in Nigerian locally adapted and exotic turkeys were analyzed by real-time PCR and calculated using $2^{-\Delta\Delta Ct}$. The highest ($p<0.05$) ΔCT (12.82) and R (1.38) were respectively found in the breast and liver tissues of local black turkey at 6 weeks while the highest ($p<0.05$) ΔCT (10.60) and R (1.24) of MSTN gene were obtained from the breast and liver tissues of exotic turkey at 16 weeks. Generally, there was reduction in relative expression at finisher phase in the breast and thigh tissues of exotic and Nigerian locally adapted (black and lavender plumage variants) turkeys. This expression pattern provided useful information on the genetic basis for growth variation in exotic and Nigerian locally adapted turkeys and could be employed for genetic improvement of Nigeria locally adapted turkeys in a short period of time.
Key words: Myostatin gene, exotic turkey, Nigerian locally adapted turkeys, Liver tissues, Breast tissue, Thigh tissue

INTRODUCTION

Turkey production is an aspect of poultry production that is now popular in Nigeria due to the high demand for turkey meat. Most of the turkeys bred in Nigeria are local turkeys, with few exotic and crossbreds (Ilori *et al.,* 2018). The large differences in growth between Nigerian locally adapted and exotic turkeys are attributed to genetic variation due to different selection methods under their evolution and environment. Myostatin gene is one of the genes that controls and regulates muscle production in animals. This gene (Myostatin) is thought to play a role in postnatal muscle growth by controlling satellite cell stagnation (McCroskery *et al*., 2003) probably because it acts by negatively regulating skeletal muscles. In men, weight loss in HIV-infected men is attributed to an increase in serum concentrations of the myostatin gene (Gonzalez–Cadavid *et al*., 1998). Similarly, the muscle multiplication of Belgian blue and Piedmont cattle, which is commonly considered to be double-muscular cattle, was associated with mutations in the Myostatin gene (Kambadur *et al*., 1997). Therefore, this study focused on the relative and differential expression of the myostatin gene in Nigerian locally adapted and exotic turkeys.

MATERIALS AND METHODS

This research work was carried out at the Turkey Breeding Unit of the Directorate of University Farms (DUFARMS) and Animal Biotechnology Laboratory of the Department of Animal Breeding and Genetics (ABG), Federal University of Agriculture, Alabata road, Abeokuta, Ogun state Nigeria. A total of two hundred turkeys comprising one hundred and fifty Nigerian locally adapted (fifty each of black, lavender and white colour variants) and fifty Hybrid converter (exotic) 1-day-old poults were used for this study. The poults were raised intensively in deep litter pen with adequate medication and feeding. All the experimental birds exposed to the same management practices throughout the experimental period of 16 weeks. Six turkeys were randomly selected from each of Nigerian locally adapted and exotic turkeys, at six weeks (grower stage) and sixteen weeks (finisher stage) of age to extract the total RNA in liver, breast and thigh muscle tissues. About 1 g of tissue samples were collected and stored in a collection tube under RNA later® until used.

The extraction of RNA was done by homogenizing the tissue samples in TRIzol reagent (Invitrogen, Carlsbad, CA) using a micro–pestle and mortal. Total RNA was extracted using Gene aid RNA extraction kit (Tissue), according to the manufacturer's protocols (Invitrogen, Carlsbad, CA). RNA concentration was determined by measuring the absorbance at 260 nm using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware). RNA quality was assessed by ensured every RNA has an absorbance of between 1.8 and 2.0 at A260/A280. The extracted RNA was converted to cDNA using the FIREScript RTcDNA Synthesis KIT. The process involved using 1 µl of Reverse Transcriptase, 2 µL of 10 x reaction buffer, 0.5 µl RNase Inhibitor (Ribogrip), 0.5 µl of primers with a 5–uM concentration and 10 µl of the RNA sample (at 50ng/µL). Nuclease Free Water was used to balance the reaction volume to 20 µl. The thermo–cycling 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 cDNA was amplified using the My IQ single colour real time cycler. The qPCRmix used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25µl reactions consisting of 4 µl of the 5x HOT Firepol qPCR Mix, 0.4 µl each of the forward (5' AGC ACC TAA CAT TAG CAG GGA CGT 3') and reverse (5' CAA AAT CTC TGC GGG ACC GT 3') primers (Shin *et al.,* 2015**)** and 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, 56 and 53°C for 20 seconds (for MSTN and GAPDH respectively) and Elongation at 72°C for 20 seconds. Melting curve analysis and gel electrophoresis was conducted after PCR amplification to ensure the primers specificity and a single PCR product. Finally, the average cycle threshold (CT) values after normalizing to GAPDH gene were used for calculating cycle threshold (ΔCT) as follows:

ΔCT = CT (MSTN) – CT (GAPDH). The relative expression of MSTN gene was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The data obtained on ΔCT of MSTN and relative gene expression (R) after normalization using logarithmic transformation, were subject to General Linear Model of SAS inc SAS, 2014. Means that are significantly different were separated using Duncan Multiple Range Test of the same software. The model is as follows: $Y_{ij} = \mu + G_i + \varepsilon_{ij}$

Where Y_{ijk} is the parameter of interest; µ is the population mean; G_i is the effect of i^{th} genotype and ε_{ijk} is the experimental error.

RESULTS

Table 1 showed the differential expression of MSTN gene in liver, breast and thigh tissues of Nigerian locally adapted and exotic turkeys at weeks 6 and 16. The ΔCT and R of MSTN gene were not significantly ($p>0.05$) affected by the tissue types in exotic turkey at week 6. The ΔCT of MSTN gene ranged from 7.15 in the thigh tissue to 10.66 in the liver tissue while the R of MSTN gene in exotic turkey ranged from 0.99 in the liver tissue to 1.32 in the breast tissue. On the other hand, there were significant ($p<0.05$) effect of different tissue type (liver, breast and thigh) on ΔCT and R of MSTN gene among the different strains of Nigerian locally adapted turkeys. The highest ($p<0.05$) ΔCT (12.82) and R (1.38) were respectively found in the breast and liver tissues of local black turkey. Also, the significantly ($p<0.05$) highest ΔCT (13.86) was recorded in the liver tissue of local white turkey while the highest ($p<0.05$) R (1.97 and 1.51) of MSTN gene were obtained in the thigh and breast tissues of local white turkey respectively. There was also significant ($p<0.05$) effect of tissue types on ΔCT and R of MSTN gene in lavender turkey. The highest ($p<0.05$) ΔCT (11.47) was recorded from the thigh tissue while the highest ($p<0.05$) R (4.93) of MSTN gene was obtained from the breast tissue of lavender turkey.

There were significant ($p<0.05$) effect of different tissue types on ΔCT and R of MSTN gene of the different turkey genotypes at week 16 of this study. The highest ($p<0.05$) ΔCT (10.60) and R (1.24) of MSTN gene were obtained from the breast and liver tissues of exotic turkey respectively. In local black turkey, the highest ($p<0.05$) ΔCT (13.96) and R (1.16) of MSTN gene were also recorded from the liver and breast tissues. Also, the breast tissue and the liver tissue respectively had the highest ($p<0.05$) ΔCT (11.84) and R (2.63) of MSTN gene in the local white turkey. The ΔCT of MSTN gene was significantly ($p<0.05$) highest (13.77) in the liver of the lavender turkey followed by the breast tissue (9.388) and 7.66 which was the least in the thigh tissue. The R of MSTN gene were higher (1.16 and 1.04) in the thigh and breast tissues of lavender turkey respectively than the liver tissue.

Table 1: Differential expression of MSTN gene in liver, breast and thigh tissues of Nigerian locally adapted and exotic turkeys at weeks 6 and 16

		Week 6		Week 16	
Genotype	Tissue	ΔCT	R	ΔCT	R
Exotic	Liver	10.66±0.074	0.99±0.022	9.85±0.088[b]	1.24±0.027[a]
	Breast	8.44±0.040	1.32±0.012	10.60±0.023[a]	`0.67±0.007[c]
	Thigh	7.150±2.741	1.31±0.825	9.01±0.006[c]	0.75±0.002[b]
Local black	Liver	9.37±0.001[b]	1.38±0.0002[a]	13.96±0.012[a]	0.002±0.004[c]
	Breast	12.82±0.191[a]	0.002±0.057[c]	8.96±0.092[b]	1.16±0.028[a]
	Thigh	7.45±0.173[c]	1.22±0.052[b]	7.93±0.029[c]	1.08±0.009[b]
Local white	Liver	13.86±0.026[a]	0.03±0.008[b]	5.22±0.0001[c]	2.63±0.0003[a]
	Breast	7.80±0.002[b]	1.51±0.001[a]	11.84±0.081[a]	0.30±0.024[c]
	Thigh	4.47±1.669[b]	1.97±0.502[a]	10.09±0.017[b]	0.42±0.005[b]
Lavender	Liver	7.56±0.029[b]	1.93±0.009[b]	13.77±0.248[a]	0.060±0.075[b]
	Breast	–3.55±0.134[c]	4.93±0.040[a]	9.388±0.035[b]	1.04±0.010[a]
	Thigh	11.47±0.082[a]	0.01±0.025[c]	7.66±0.012[c]	1.16±0.003[a]

[a,b,c] Means in the same column and group with different superscripts are significantly different ($p<0.05$)

DISCUSSION

The delta CT values of mRNA obtained from the liver, leg and breast tissues of Nigerian locally adapted and exotic turkey showed variations of mRNA products of MSTN gene in the different tissues considered among the turkey genotypes. More so, since the higher the ΔCT, the lower the rate of gene expression (Mimmack *et al.,* 2002), therefore, the higher ΔCT of MSTN gene obtained at grower phase among Nigerian locally adapted turkeys indicates the lower expression of MSTN gene in those tissues. Although, the ΔCT of MSTN gene was relatively low in exotic turkey at grower and finisher phases, the least values of ΔCT of MSTN gene in various tissues sampled were obtained in Nigerian locally adapted turkeys. This explains the differences in growth patterns in exotic and Nigerian locally adapted turkeys. This, by implication suggests comparable growth rate in Nigerian locally adapted and exotic turkey at grower phase probably because the former were in their native environment while the later was taking time to adjust to the new environment. Generally, overexpression of MSTN in the tissue is associated with reduced total cell number and inhibited differentiation (McCroskery *et al.,* 2003) hence, the higher expression of MSTN gene (as shown by the least values of ΔCT of MSTN gene) could be responsible for relatively smaller body size of Nigerian locally adapted turkeys at finisher phase. On the other hand, the exotic turkey with moderately low ΔCT values of MSTN gene in liver, breast and thigh tissue is generally noted for higher growth rate and heavy body weight. According to Bhattacharya *et al.* (2015), the expression of MSTN gene is always lower in meat type chicken than in layer type chicken. Worthy of note is the ΔCT values of MSTN gene which increased higher at 16 weeks than at 6 weeks of age in the liver tissue of Nigerian locally adapted black and lavender plumage colour turkeys. This implies that MSTN expression in different tissues may be age dependents, although no analytical result is provided in this study to explain this pattern, however, early period growth is very important as morphogenesis in particular formation of skeletal muscle, brain and digestive tract start during early stage of growth and development (Saxena *et al.,* 2007).

This result indicates that differences exist in the MSTN mRNA expression among the different plumage colour variants of Nigerian locally adapted turkeys and various tissues in the body. Lv *et al.* (2015) likewise obtained similar results when compared the MSTN mRNA expression in the different muscle of sheep. Since MSTN gene expression is inversely related to myogenesis, therefore, the differential tissue expression of this gene at grower and finisher phases may suggest variation in the role it plays in different tissue at different stages of growth of these turkey populations. Similar pattern of relative expression of MSTN gene was reported by Kuang *et al.* (2014), who observed that at age of 84 days, ZIKA rabbits contained significantly lower MSTN mRNA level in both *longissimus*

dorsi and biceps femoris muscles than Californian rabbits and mRNA levels of *MSTN* exhibited opposite changes from the age of 35 to 84 days.

CONCLUSION

The MSTN gene expressed differently at different growth phases and in different tissues and genotypes of exotic and Nigerian locally adapted turkeys. This expression patterns provided useful information on the genetic basis for growth variation in exotic and Nigerian locally adapted turkeys. Therefore, this knowledge could assist in achieving genetic improvement of Nigerian locally adapted turkeys using genomic tools within a short time.

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