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Mitochondrial Coding Gene Polymorphisms Associated with Carcass Traits in Beijing Duck

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Abstract: PCR-SSCP and DNA sequencing was employed to analyze polymorphisms of the mitochondrial coding gene including ND1-6, ND4L, COX 1-3, ATP6 and ATP8 in Chinese Beijing duck and analyze their effects on carcass traits in Beijing duck. The results showed that 4 SNPs (G9259A, T9269C, G9394A and A9601G) were detected in ATP6 gene and 1 SNP (G9902A) was detected in COX 3 gene. The G9902A locus was a nonsynonymous which result in the varation of the coding amino acid (Arg56Gln). The COX 3 locus showed three genotypes (c. 9902GG, c. 9902GA and c. 9902AA) and the frequencies were 0.12, 0.23 and 0.65, respectively. The COX 3 locus was significantly associated with carcass traits of Beijing duck ($p<0.05$).

Key words: Mitochondrial DNA, carcass traits, COX 3, duck, significantly, polymorphism, sequencing

INTRODUCTION

Mitochondria are the site of energy production in the cell and produce the majority of cellular ATP so the variations in poultry growth performance and phenotypic expression of feed enfficiency could be due in part to inefficiencies in mitochondrial function (Bottje *et al*., 2002; Havenstein *et al*., 2003). The activities of mitochondrial respiratory chain complexes in breast muscle of broiler chickens from high-efficiency genetic lines increased compared to low-efficiency lines (Iqbal *et al*., 2004). Likewise, greater mitochondrial complex activity was observed in liver (Iqbal *et al*., 2005) and lymphocytes of high versus low feed efficient lines of broiler chickens. Similarily, higher muscle mitochondrial activity in Angus steers exhibiting higher feed efficiency compared to steers with lower feed efficiency (Connor *et al*., 2009).

Mitochondria contain their own DNA (mtDNA), which encodes a small but essential subset of the oxidative phosphorylation (OXPHOS) machinery responsible for the production of ATP, the remaining subunits of which are encoded by nuclear DNA (Shadel and Clayton, 1997). The mitochondrial electron-transfer chain is composed of 5 enzyme complexes (complexes I-V). Four of these 5 complexes (I, III and IV) contain subunits encoded by mtDNA. The NADH-CoQ reductase contain 7 subunits encoded by mtDNA (ND1-6, ND4L). The three largest subunits of cytochrome c oxidase (Complex IV), COX 1-3 are encoded in the mitochondrial DNA (mtDNA) and form the catalytic core of the enzyme. Mitochondrial ATP synthase contain 2 subunits (ATPase subunits 6 and 8) encoded by mtDNA.

The mutations in mtDNA can change OXPHOS whereby affect the energy metabolism. A number of evidence support that mitochondrial variants contribute to diabetes risk and some mitochondrial variants have been posited as being associated with type 2 diabetes (Stark and Roden, 2007). In livestock, the associations of polymorphisms of mitochondria DNA with economic traits have been observed.

The mitochondria displacement loop (D-loop) polymorphisms were associated with a negative effect on milk composition traits. Likewise, mitochondria D-loop and 16S rRNA variations have strong association with longissimus muscle area and beef marbling score of Japanese Black cattle. Recently, a haplotype of the ND5 gene was detected to have a positive effect on growth traits at 6 months in the Chinese Nanyang breed (Zhang *et al*., 2007). Two polymorphic positions detected in H3 haplotype, a synonymous transition 9104C>T in the gene-coding region of Cytochrome c oxidase subunit III and a substitution 715A>G in 12 S rRNA were associated with longissimus muscle composition in Iberian pigs (Fernandez *et al*., 2008). These indicate that there may exist significant DNA marker for meat quality traits selection on mitochondrial DNA.

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The objective of this study was to evaluate the variations of mtDNA coding regions (including ND1-6, ND4L, COX 1-3, ATP6 and ATP8) and their effects on carcass traits in Beijing duck.

MATERIALS AND METHODS

Samples collection and preparation: The blood samples 285 ducks were collected from Institute of Animal Science in Beijing. All birds were raised in floor pens and fed commercial corn-soybean diets that met NRC requirements and were slaughtered with appropriate humane methods at 42 days of age. Body weight, carcass yield, chest muscle weight, leg muscle weight, subcutaneous fat plus skin weight, abdominal fat weight, chest muscle ration, leg muscle ration, abdominal fat ration and percentage of subcutaneous fat plus skin weight were measured. Genome DNA was obtained by phenol and chloroform (1:1) extraction and stored at -20°C.

Primer design and PCR amplification: The PCR primers (Table 1) were designed to amplify duck most mtDNA coding regions (EU755252) by using Primer V5.0 software. The PCR products covered almost all the coding sequences of duck mtDNA except cytb. The 20 μL PCR reaction volume contained 50 ng DNA template, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Dingguo, Beijing, China). The PCR protocol was performed as the following program: 94°C for 5 min followed by 35 cycles of 94°C for 40 sec, annealing for 40 sec and 72°C for 1 min and a final extension at 72°C for 10 min.

Single Stranded Conformation Polymorphism (SSCP): Aliquots of 5 μL of above PCR products were mixed with 5 μL of the denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to 10% PAGE (80×73×0.75 mm) analysis which was run with 1×TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) for 2 h at room temperature under a constant voltage (150 V). The gel was stained with silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde) according to Zhang *et al*. (2007).

DNA sequencing analysis: The PCR products amplified by each primer pairs from a DNA pool (the mix of the 285 genomic DNA) were sequenced to detect all SNPs of 12 genes. For the non-synonymous Single Nucleotide Polymorphisms (SNPs), the PCR products from different PCR-SSCP genotypes were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich Corporation, USA) and sequenced by the ABI 3730 sequencer from both directions (Applied Biosystems, USA). In a specific locus, the PCR products that represented different PCR-SSCP genotypes were sequenced so several SNPs may be found in a single PCR product. The mutations appeared simultaneously in the individuals with specific PCR-SSCP genotypes.

Table 1: Primer pairs used for PCR amplification of the mitochondrial DNA coding gene

Gene	Primer sequences	Anneal temperature (°C)
ND1	5'-TTC CCT CTA AGT AGT GAA ACC A-3'	62.0
	5'-TTT GAG TTC AGG CAC ATT TC-3'	
ND2	5'-TAG TCC TCA GTC TCG CAT TAG-3'	63.0
	5'-GTG TCC TGT TGG TCT TAG CG-3'	
ND3	5'-TTC CAC TTC ACA TCA GAC CAC CAC-3'	66.5
	5'-GGG CGA CAT AAG AAA GTT ACA GG-3'	
ND4	5'-ACT CAG CCT TCA TTC TTA GCG-3'	65.0
	5'-GAT TAG TTC GGG CTT GAG GA-3'	
ND4L	5'-TAC CCT GGG CAA TCC AAC TA-3'	66.0
	5'-AGT CAG CAG GAT AGC ACC AG-3'	
ND5	5'-CTA CTA ACC GCA TCA TAC ACC T-3'	65.0
	5'-GTG TAT GCG GAT AAG TAG GAA G-3'	
ND6	5'-CCG CCT CCG TCC TAA TCC TA-3'	67.0
	5'-GCC GTA GGT CTC GGG TAG TG-3'	
COX1	5'-GTT CGG CAA CTG ATT GGT CC-3'	65.0
	5'-ACA ATC AGA GCG TGG TCG TG-3'	
COX2	5'-CTG TGA AAC ACC GTC TCC TC-3'	65.5
	5'-TAG GAT GAC GAT AGG AGG GA-3'	
COX3	5'-CAG CCT CCA TCG CAC TCA TA-3'	64.0
	5'-TCC GCA TTC GTA TGG TGA GA-3'	
ATP6	5'-TGA ATC AAC AAC CGA CTA TCC-3'	62.5
	5'-TAG GAG CAT TGA TAA GAG GC-3'	
ATP8	5'-TCA TCC CTC CTA TCG TCA TC-3'	60.0
	5'-GGC AGT AAG TGA GCC AAG GA-3'	

Statistics: Association analyses between the SNPs and traits in 285 ducks were performed using SPSS 13.0 with the following model:

$$Y = \mu + G + e$$

Where:

Y = The dependent variable (analysed traits)
μ = The overall mean
G = The genotype of COX 3 (c. 9902GG, c. 9902GA or c. 9902AA)
e = The random error

RESULTS AND DISCUSSION

The SSCP polymorphisms in duck COX 3 and ATP6 gene were detected. No SNP was detected in ND1-6, ND4L, COX 1-3 and ATP8 gene. The PCR products of different electrophoresis patterns were sequenced. Based on the reference sequence (EU755252), five novel SNPs were found. The SNP G9259A, T9269C, G9394A and A9601G located on ATP6 gene and G9902A located on

Table 2: Effects (least-square means) of G9902A genotypes on Carcass traits of Beijing ducks at 42 days

Carcass traits	Genotype		
	c. 9902AA (185)	c. 9902GA (66)	c. 9902GG (34)
BW (g)	3014.2±22.7[a]	2910.6±43.1[b]	2860.9±47.6[c]
CY (g)	2531.5±19.8[a]	2449.2±35.6[b]	2408.8±39.2[b]
CMW (g)	246.1±4.40[a]	224.1±7.30[b]	212.4±9.50[c]
LMW (g)	291.2±3.20	288.9±6.20	287.6±7.70
SW (g)	797.9±10.5[a]	744.4±17.3[b]	718.5±20.9[c]
AFW (g)	60.6±1.40[a]	55.1±1.80[b]	52.1±2.70[b]
CMR (%)	9.7±0.10[a]	9.1±0.20[b]	8.9±0.30[b]
LMR (%)	11.5±0.10	11.8±0.20	11.9±0.20
AFR (%)	2.4±0.10	2.3±0.10	2.2±0.10
SR (%)	31.5±0.30[a]	30.3±0.40[b]	29.8±0.40[b]

BW, Body Weight; CY, Carcass Yield; CMW, Chest Muscle Weight; LMW, Leg Muscle Weight; SW, Subcutaneous fat plus skin Weight; AFW, Abdominal Fat Weight; CMR, Chest Muscle Ration; LMR, Leg Muscle Ration; AFR, Abdominal Fat Ration; SR, percentage of Subcutaneous fat plus skin weight. The number in bracket is the total number of birds with specific genotype

COX 3. The 9902G>A resulted the variation of 56 Arg→Gln in COX 3. The variation was evaluated by a web based (http://cubic.bioc.columbia.edu/services/snap/submit.html) software SNAP (Bromberg and Burkhard, 2007) and the result indictaed that it was a non-neutral variation which may affect the function of COX 3. The change from charged amino acid (Arg) to no-charged amino acid (Gln) changed the hydrophilicity of COX 3 residues so that affected the stability of COX 3 peptide (Strub *et al*., 2004; Chasman and Adams, 2001). So we assumed this variation was associated with the enenrgy utilization of duck and affected the production performance of duck.

The SNP 9902G>A was genotyped by PCR-SSCP. The frequency of the three genotypes was 0.12 (c. 9902GG), 0.23 (c. 9902GA) and 0.65 (c. 9902AA), respectively. The c. 9902AA is the predominant genotype. Significant differences among least-square means of the three genotypes were analysed using a contrast test. The results revealed that there were significant differences among homozygotes (c. 9902GG and c. 9902AA) and heterozygotes (c. 9902GA) for body weight, carcass yield, chest muscle weight, subcutaneous fat plus skin weight, abdominal fat weight, chest muscle ration and percentage of subcutaneous fat plus skin weight of Beijing duck at 42 days (Table 2). The genotype c. 9902AA had postive effect on the traits above metioned.

Mitochondria is responsible for genetic variation in cytoplasmic effects since they contained maternally inherited DNA. Direct heritability estimates for the 8 weeks weight, scan weight, muscle depth and fat depth of sheep using the full dataset were 0.18, 0.25, 0.24 and 0.21, respectively (Pritchard *et al*., 2008). The mitochondria DNA variations have been reported to be strong associated with the carcass traits of cattle and pig Fernandez *et al*., 2008). In present study, the COX 3 gene G9902A SNP was associated with the carcass traits of duck. This further confirmed genetic importance of mitochondria DNA.

CONCLUSION

The duck mitochondria DNA region was polymorphic. The COX 3 gene was significantly associated with carcass traits of duck so the single nucleotide deletion polymorphism of COX 3 could be an important genetic marker for carcass traits and can be potentially used in duck breeding.

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