IJAS Iranian Journal of Applied Animal Science

# The Effect of Uncoupling Protein Polymorphisms on Growth, Breeding Value of Growth and Reproductive Traits in the Fars Indigenous Chicken

Research Article

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Received on: 3 Jun 2016
Revised on: 19 Sep 2016
Accepted on: 1 Oct 2016
Online Published on: Dec 2017

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Online version is available on: www.ijas.ir

## ABSTRACT

The avian uncoupling protein (avUCP) is a member of the mitochondrial transporter superfamily that uncouples proton entry in the mitochondrial matrix from ATP synthesis. The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used to estimate the allele and genotype frequencies of the UCP/*HhaI* polymorphisms and to determine associations between these polymorphisms and the growth traits, breeding value of growth and reproductive traits in the Fars indigenous chicken. For this purpose phenotype information of 18 successive generations from 200 birds were analyzed using a univariate animal model in ASREML procedure. The evaluation of the association between this single nucleotide polymorphisms (SNP) with reproductive traits suggests a positive effect of TC genotype with age at first egg (ASM) compared with CC genotype. In addition, TC genotype was significantly associated with the breeding value of age at first egg compared with the CC genotype ($P<0.05$). In conclusion, our results suggest that the TC genotype of the UCP gene is associated with age at sexual maturity (ASM) and breeding value of age at sexual maturity and UCP polymorphisms may be used as DNA markers for selection in the breeding process of the Fars indigenous chicken.

KEY WORDS Fars indigenous chicke, PCR-RFLP, polymorphism, reproductive trait, uncoupling protein.

## INTRODUCTION

Strategies in poultry breeding programs aim to increase feed efficiency, growth rate, and body weight (BW); decrease abdominal fatand production costs. Low energy expenditure, in addition to increased energy intake, has been a major cause of future weight gain, and variations in energy expenditure may be one of the underlying sources of variation in BW (Saltzman and Roberts, 1995). Various determinants, including body composition, hormonal levels, activity of the sympathetic nervous system, and genetics are responsible for differences in metabolic rate among individuals (Toubro *et al*. 1996). A mitochondrial protein called uncoupling protein plays an important role in generating heat and burning calories by creating a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondrial membrane in brown adipose tissue, without coupling to any other energy-consuming process. This pathway has been implicated in the regulation of body temperature, body composition, and glucose metabolism (Himms-Hagen, 1990). The uncoupling protein (*UCP*) gene is one of the many genes that might be related to the growth traits, breeding value of growth and reproductive traits. The UCP3 is the member of UCPs family of the mitochondrial transporters, all of which are known to uncouple oxidative phosphorylation via proton leakage from the inner mito-

chondrial membrane. The thermoregulation and energy metabolism are potentially regulated by these mitochondrial proteins (Adams, 2002). The UCP is a candidate gene for growth traits, breeding value of growth and reproductive traits because it may contribute to the proton leak that occurs in mitochondria and partially uncouples the oxidative phosphorylation, thus releasing chemical energy as heat (Krauss *et al*. 2002). The *UCP* gene family involved in energy metabolism of body, and had significant effects on energy balance related traits, such as BW basal metabolic rate, efficiency of feed conversion, and etc. (Ricquier, and Bouillaud, 1997). Motloch *et al*. (2016) suggested that beyond the UCP2, UCP3 also exhibits regulatory effects on cardiac mCa1/MCU function. in addition, the UCP3 is associated with Hax-1 in mitochondria in the presence of calcium ion (Hirasaka *et al*. 2016). Liu *et al*. (2005) found the *UCP3* gene polymorphisms may contribute to body mass index (BMI) in the Caucasian population. Rudofsky *et al*. (2006) found that the human *UCP3* gene involved in the energy balance and is associated with obesity and diabetes. In human, the *UCP2* gene is expressed extensively in various tissues, like white adipose tissue, pancreas, skeletal muscle, and liver (Gimeno *et al*. 1997). Both environmental and genetic factors may have contributed to the the susceptibility of the growth traits, breeding value of growth and reproductive traits. Genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of the specific breed. Therefore, it is important to try to characterize genetically indigenous breeds (Shojaei *et al*. 2011). A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites and also the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity it contains. Therefore, studies of the population genetic diversity and the structure of population within and between species may not only illustrate the evolutionary process and mechanism but also provide information useful for biological conservation of the Bovidae family, sheep, goats, birds and so on (Askari *et al*. 2011). The molecular markers are increasingly used for the study of the genetic diversity of populations in recent years (Zamani *et al*. 2013; Zamani *et al*. 2015). Archaeological excavations confirmed the presence of the domestic fowl in the territory of Iran at the ancient times (Mohammadabadi *et al*. 2010). It is known that Persian chickens from the Gilan province took part in the origin of the Russian Orloff breed (Mohammadabadi *et al*. 2010). Since 1981, twelve chicken breeding centers were established for reproducing native poultry varieties, and a total number of chickens they maintain are about 8000 birds. Currently, there are eight breeding centers in Fars, West Azarbaijan, Isfahan, Mazandaran, Khorasan, Yazd, Zanjan and Khuzestan provinces (Mohammadabadi *et al*. 2010). Research on native chicken populations of Iran has been initiated, and the data on the genetic variability of different loci in these populations have been published (Mohammadabadi *et al*. 2010; Mohammadifar *et al*. 2013; Moazeni *et al*. 2016). However, data on genetic variability of UCP locus in Iranian native chickens, especially in Fars indigenous chicken have not been published. Therefore, the objective of this study was to investigate association of polymorphism of *UCP* gene with growth traits, breeding value of growth and reproductive traits in subjects from Iran.

## MATERIALS AND METHODS

Breeding station of Fars indigenous chicken is located at Shamsabad (Perspolis) 70 km far from north of Shiraz, the provincial capital of Fars state, located in the south of Iran. In 1986 about 4000 cocks and hens were purchased from rural regions across the Fars province and kept in a quarantine farm for a year. From those, about 400 birds of two sexes were kept to produce hatching eggs and chicks produced from these eggs were transferred to the station. Since then the birds have been individually tagged and trap nest has been used for pedigree recording. Parents of each generation (about 100 cocks and 500 hens) are selected among 5000 pedigreed and performance recorded birds produced each generation.

Blood samples were collected from the chicken, including 15 males and 185 females belonged to generation 17. Approximately, 1 mL of blood samples was obtained from the wingvein and collected in ethylenediaminetetraacetic acid (EDTA) contained tubes. The genomic DNA was extracted from white blood cells using standard salting out procedure described by Abadi *et al*. (2009). The DNA samples were dissolved in TE (Tris-EDTA) buffer which was made from 10 m*M* Tris–Cl (pH=7.5) and 1 m*M* EDTA (pH=8.0) and were stored at 20 ˚C for use.

Gene specific primers were designed according to the DNA sequence of the UCP (Accession:AF433170) using the oligonucleotide design tool Primer 5.0 software (F:5'TACCCCAAGCATGCAGAACTCA-3' and R:5'GGAACCGCACCTTGACCAC-3').

Twenty five microliters of PCR reaction mixtures consisting of 75 to 100 ng of chicken genomic DNA (1 µL) in 1× PCR reaction buffer (2.5 µL) (supplied by the manufacturer), 3 m*M* $MgCl_2$ (1.5 µL), 0.25 µ*M* of each primer (0.6 µL), 0.2 m*M* of each dNTP (2 µL), *Taq* DNA

polymerase Promega (0.2 µL) (Promega, Madison, WI) and 16.6 µL $dH_2O$ with the following conditions: 94 ˚C for 5 min, 35 cycles of 94 ˚C for 30 s, annealing at 55 ˚C for 60 s, 72 ˚C for 60 s, and a final extension at 72 ˚C for 7 min. PCR products were electrophoretically separated on 2% agarose gel (5 V/cm), stained with ethidium bromide. PCR products were digested with 10 units of *HhaI* restriction enzymes (Fermentase, Lithuania), 6 mL of PCR product, 1.4 mL of Tango buffer and 2 mL nuclease-free water. The final volume of 10 mL was incubated in 37 ˚C for 12 h. The fragments were separated a 3.5% agarose gel stained with ethidium bromide.

Whole information data file (18 successive generations) consisted of three fixed effects (generation, sex and hatch) and 11 recorded traits, including BW at hatch (BW1), BW at ages of 8 (BW8), 12 (BW12) weeks, BW at sex maturation (WSM), age at first egg (ASM), egg number (EN), first egg weight (EW1), average egg weight at ages of 28 (EW28), 30 (EW30) and 32 (EW32) weeks and average egg weight for the first 12 weeks of production (EW12). The BW1, BW8 and BW12 have been measured in both male and female chicken. Also, three combined traits consisting of the average of EW28, EW30 and EW32 (AV), intensity of egg production (EINT=(egg number/days recording)×100) and egg mass (EM=EN×EW12) were calculated to use in the analyses (Table 1). During 18 generations, genetic evaluation of the birds for BW at 8 weeks, age of the hens at first egg, average egg weight and total number of eggs laid during first 12 weeks after flocks maturity (when 5% of the flock are in egg production) have been performed. Economic indices are calculated for these traits and birds of two sexes are selected based on their aggregate genotypes for these traits. The goals of the breeding station on the one hand are to increase BW, egg weight and egg number and on the other hand, to decrease age at the first egg.

Pedigree and data file were prepared using Visual FoxPro 9.0 software, the relational data base management system. SAS 9.1 package was used to carry out the descriptive statistics and fitting model (SAS, 2002). The fixed effects and their interactions were considered in the animal model that provided having significant effect. Genetic analyses were performed using ASREML software (Gilmour *et al*. 2006). The breeding values of growth and egg production traits were estimated using the BLUP based on an animal model with a relationship matrix. The used models in matrix notation were as follows:

$$y= Xb + Za + e$$

Where:
y: vector of observations.
b: vector of the fixed effects of generation, sex and hatch.
a: vector of random direct genetic effects.
e: vector of random residual effects.
X and Z: incidence matrices relating the observations to the respective fixed and direct genetic effects.

Calculation of gene frequency carried outbased on direct gene count method by f(A)= (2nAA+nAa)/2n or f(G)= (2nGG+nGg)/2n, and the standard error of frequency was calculated as: (p(1- p)/2n)1 / 2

Where:
n: sample size.
p: frequency of A or G allele.

Marker-trait association analyses were conducted using the following modelin GLM procedure of SAS 9.1 software (SAS, 2002). The significant differences of least squares means were tested with Tukey–Kramer's multiple range tests, and a P-value of ≤ 0.05 was considered statistically significant.

$$Y_{ijk}= \mu + M_i + e_{ijk}$$

$Y_{ijk}$: estimated breeding values of the trait.
µ: population mean.
$M_i$: fix effect of genotypes.
$e_{ijk}$: residual random error.

There was no significant interaction between the gene's additive effects.

## RESULTS AND DISCUSSION

Statistical description of data set for growth and egg production traits based on *UCP* gene is shown in Table 1. A single nonsynonymous SNP was a C > T substitution at position 1270 in exon-3 replacing Ala > Val. The length of PCR product was 222 bp. A PCR-RFLP test using *HhaI* restriction enzyme was designed to screen the *UCP* Ala118Val polymorphism (Table 2).

The digested allele and the undigested allele were identified as allele T and allele C, respectively. Three genotypes TC (Ala/Val), TT (Ala/Ala) and CC (Val/Val), were detected after the PCR-RFLP analysis of the *UCP* gene. The allele frequencies were 0.25 for T and 0.75 for C, and the genotypic frequencies were 0.20, 0.15, and 0.65 for TC, TT and CC, respectively. The association between the *UCP* genotypes and growth and egg production traits are shown in Table 3 and between the *UCP* genotypes and breeding values of growth and egg production traits are given in Table 4.

**Table 1** Statistical description of data set for growth and egg production traits

| Traits | No. of animal | Mean | Coefficient of variation |
|---|---|---|---|
| BW1 (g) | 34.51 | 34.38 | 9.21 |
| BW8 (g) | 42.76 | 559.9 | 16.98 |
| BW12 (g) | 38.12 | 952.8 | 14.01 |
| WSM (g) | 31.01 | 1689 | 11.52 |
| ASM (day) | 31.12 | 164.9 | 8.99 |
| EN (number) | 30.99 | 36.01 | 38.89 |
| EW1 (g) | 26.89 | 40.99 | 15.02 |
| EW28 (g) | 17.03 | 45.99 | 8.04 |
| EW30 (g) | 18.99 | 47.96 | 8.14 |
| EW32 (g) | 18.06 | 48.78 | 8.12 |
| EW12 (g) | 18.01 | 46.05 | 9.02 |
| AV (g) | 27.77 | 45.54 | 12.93 |
| EM (g) | 28.06 | 1767 | 38.97 |
| EINT (%) | 30.79 | 56.94 | 32.95 |

BW1: body weight at birth; BW8: body weight at 8 weeks of age; BW12: body weight at 12 weeks of age; WSM: weight at sexual maturity; ASM: age at first egg; EN: egg number; EW1: weight of first egg; EW28: average egg weight at 28 weeks of age; EW30: average egg weight at 30 weeks of age; EW32: average egg weight at 32 weeks of age; EW12: average egg weight for first 12 weeks of production; AV: average for EW28, 30 and 32; Egg mass (EM)= EN × EW12 and Egg production intensity (EINT)= (egg number/days recording) × 100.

**Table 2** Summary of variations in uncoupling protein (*UCP*) gene

| Location | Position | Nucleotide changes | Amino acid changes | Subtype |
|---|---|---|---|---|
| Exon 3 | 1270 | C > T | Ala118Val | Missense |
| | 1316 | T > C | Cys133Cys | Silent |

**Table 3** Association of the uncoupling protein (UCP) genotypes at the growth and egg production traits (Mean±SE)

| Traits | Genotype TT | Genotype TC | Genotype CC |
|---|---|---|---|
| WSM (g) | 1794.89±34.99 | 1841.79±56.81 | 17210.68±19.97 |
| ASM (day) | 184.77±3.44$^{ab}$ | 188.96±4.85$^{a}$ | 175.95±1.67$^{b}$ |
| EN (number) | 39.01±1.30 | 42.10±2.12 | 39.94±0.76 |
| EW28 (g) | 47.79±0.83 | 48.03±1.41 | 46.99±0.39 |
| EW30 (g) | 48.99±0.70 | 49.48±1.25 | 48.98±0.40 |
| AV (g) | 49.87±0.62 | 50.99±1.10 | 49.45±0.33 |
| EM (g) | 1980.22±69.65 | 2156.83±116.73 | 1984.99±40.11 |
| EINT (%) | 62.01±2.02 | 64.98±3.73 | 62.57±1.29 |

WSM: weight at sexual maturity; ASM: age at first egg; EN: egg number; EW28: average egg weight at 28 weeks of age; EW30: average egg weight at 30 weeks of age; AV: average for EW28, 30 and 32; Egg mass (EM)= EN × EW12 and Egg production intensity (EINT)= (egg number/days recording) ×100.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

The missense mutations of *UCP* gene, were significantly linked to ASM (P<0.05) and also highly significantly linked to breeding value of ASM (P<0.05).

In studied population, the ASM was higher in the heterozygote (UCP Ala/Val) in compared with the homozygote (UCP Val/Val).

The *UCP* gene involved in regulation of energy metabolism, has important physiological effects on economical important traits of farm animals.

The gene polymorphisms can be used for improvement of the production traits by genetic selection, if the allelic association with the traits be determined. The chicken *UCP* gene was first discovered by Raimbault *et al.* (2001). Its function is still not clear, though there has been some research about it since its discovery.

The nucleic acid sequence of avian UCP is highly homologous to both mammalian UCP2 and UCP3 (Evock-Clover *et al*. 2002).

Researchers documented that the function of these two UCPs involves regulation of the fatty acid oxidation, especially during metabolic stress (Dulloo and Samec, 2001), maybe by transporting fatty acid ions from the mitochondria (Himms-Hagen and Harper, 2001). Two significant associations (P<0.05) were observed with growth, breeding value of growth and reproductive traits in this study (Tables 3 and 4). Sherman *et al.* (2008) observed significant association between a SNP (A/G) in bovine *UCP3* gene intron-3 and average daily gain and partial efficiency of growth in Continental 9 British hybrid beef steers. Han (2008) reported the existence of three SNPs (G/A, C/T and G/T) in exon 3 and four genotypes in Chinese Qinchuan cattle. Association studies showed that the genotype AA has greater slaughter weight, carcass weight, carcass length, eye muscle area, water holding capacity, and marbling than other genotypes; the genotype AB has greater back fat than other genotypes.

Table 4 Association of the uncoupling protein (UCP) genotypes on breeding values of growth and egg production traits (Mean±SE)

| Traits | Genotype TT | Genotype TC | Genotype CC |
|---|---|---|---|
| WSM (g) | 12.88±3.99 | 21.85±5.97 | -23.12±5.96 |
| ASM (day) | -22.01±0.99$^{ab}$ | -20.12±1.73$^{a}$ | -25.14±0.68$^{b}$ |
| EN (number) | 13.88±0.26 | 13.79±0.44 | 13.97±0.16 |
| EW28 (g) | 0.83±0.12 | 0.95±0.30 | 0.93±0.75 |
| EW30 (g) | 0.57±0.87 | 0.37±0.92 | 0.57±0.14 |
| AV (g) | 1.28±0.16 | 1.48±0.25 | 1.23±0.09 |

WSM: weight at sexual maturity; ASM: age at first egg; EN: egg number; EW28: average egg weight at 28 weeks of age; EW30: average egg weight at 30 weeks of age and AV: average for EW28, 30 and 32.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Li (2006) found the allele A of UCP3/*BglI* polymorphism has greaterfrequencies than allele B in Nanyang, Luxi, and Yanbian cattle. Association analysis showed the superiority of the AA genotype over the AB genotype for the content of b-globin in Luxi cattle. Fasting, which is associated with a major reduction in total body energy expenditure, is associated with an increase in the expression of UCP2 and UCP3 (Boss *et al*. 2000). However, it was also reported that *UCP2* and *UCP3* genes were unlikely to have a substantial effect on variation in obesity phenotypes in a particular US Caucasian human population (Guo *et al*. 2005).

Contreras *et al*. (2016) reported that UCP2 deficiency results in an unsuspected metabolic shift in the brain, as revealed by the finding of a significant decrease in label incorporation into glutamate, and downstream related metabolites and Chaudhuri *et al*. (2016) found that UCP2 regulates metabolic reprogramming and fate of antigen‐stimulated CD8+ T cells. Researchers also have indicated that in the context of HCC, *miR-214* acts as a putative tumor suppressor by targeting UCP2 and defines a novel mechanism of regulation of UCP2 (Yu *et al*. 2016). In animal and human studies, the UCP2 mRNA levels have been correlated with plasma-free fatty acid concentrations (Argyropoulos and Harper, 2002). In adipose tissue of obesity-resistant mice, theUCP2 expression is increased 2-fold when compared with obesity-prone animals (Fleury *et al*. 1997).

Independent studies have identified quantitative trait loci for obesity or body fat in mice that coincide with the genomic location of the *UCP2* gene (York *et al*. 1999; Taylor and Phillips, 1996) and Wang *et al*. (2016) reported that UCP2 increases susceptibility to lipopolysaccharide-induced acute lung injury in mice.

The SNP in the exon included synonymous and nonsynonymous polymorphisms. At any given position in a DNA sequence, a nucleotide can be substituted by any of the 4 nucleotide bases and may result in biallelic SNP. This occurs due to the low substitution rate of single nucleotides, estimated to be between $1 \times 10^{-9}$ and $5 \times 10^{-9}$ per nucleotide per year at neutral positions in mammals (Vignal *et al*. 2002).

Based on these numbers, the probability of 2 independent base changes occurring at a single position is very low (Vignal *et al*. 2002). A large variation in *UCP* gene sequences in the studied population was found to reside in the first half of the gene. In the present study, a missense polymorphism identified in the *UCP* gene at Ala118Val can be predicted to occur in the transmembrane region of the second domain close to the mitochondrial inner membrane space. The various biological functions of UCP are not known in detail, and based on the localization and nature of the codon UCP118 variant, it is difficult to predict if the encoded protein change may cause defects in the functionality of the UCP. Structural-functional studies are thus needed to elaborate on the effect of the UCP Ala118Val variant on theUCP's protein structure as well as function. In a highly selected commercial population, changes in the allele and genotypic frequencies between generations may result from the net reproductive success of the individuals selected as breeding stock with the different genotypes and the selection norms adopted by the breeder. Variable associations of the identified polymorphisms may be a result of the differences in the population characteristics, sex, or both, indicating that the selection criteria may influence the production trait associations. This should be taken into consideration while selecting for the desired reproductive traits.

## CONCLUSION

Additional studies are required to expand the genetic, physiological, behavioral aspects involved in feed intake, digestion and metabolism. The genomic diversity also has important implications in the evolutionary dynamics of species. Investigations of polymorphisms are useful for better understanding of the gene function, and those associated with commercially significant growth, breeding value of growth and reproductive traits have a potential for usage as molecular markers for selection programs. The identified polymorphisms and their associations with the traits of economic importance in the present study provides greater insight into the role of gene involved in energy balance in

poultry (UCP) and points toward the potential application of the findings for the enhancement of production traits by marker assisted selection.

## ACKNOWLEDGEMENT

The authors wish to thank Payamnoor University of Yazd, Iran, for their help.

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