INFLUENCE OF THE A286G POLYMORPHISM IN THE LEPR GENE ON CARCASS TRAITS IN A PATERNAL BROILER LINE

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ABSTRACT
Leptin plays a key role in the regulation of the energy balance in mammals. Thus, the leptin and leptin receptor are being intensively studied in these animals. However, it remains unclear whether the leptin-mediated control mechanism is conserved in non-mammalian vertebrates such as the avian. In this study, the association of the SNP LEPR A286G with carcass traits was investigated in the EMBRAPA TT Reference Population. A total of 1411 broilers was evaluated for 32 carcass traits. Association analyses were performed using QxPaK software with a model including fixed effects of sex, hatch and SNP, and the infinitesimal and residual random effects. Additive and dominance effects of the SNP were tested including their interaction with sex. The SNP showed significant association with carcass weight (P=0.54E-03), weights of head (P=0.28E-04), feet (P=0.41E-02), neck (P=0.44E-02), wings (P=0.20E-04), middle joint wings (P=0.27E-03), wing sticks (P=0.41E-03), thigh (P=0.11E-01), thigh muscle (P=0.12E-01), breast (P= 0.48E-04), breast muscle (P=0.18E-04), breast skin (P=0.43E-01), breast fillet (P= 0.32E-03), back weight (P=0.19E-01), drumstick yield (P=0.28E-01), breast muscle yield (P=0.11E-01), thigh and drumstick yield (P=0.95E-02), and thigh and drumstick muscle yield (P=0.72E-02). These associations are explained by the additive effect of the SNP. The results indicate that the LEPR SNP is a potential genetic marker to improve carcass traits in chickens.
KEYWORDS: candidate gene, leptin, single nucleotide polymorphism

INTRODUCTION
Leptin, a polypeptide hormone secreted mainly by adipose tissue, plays a key role in feed intake, regulation of the energy balance and reproduction in mammals (Zhang *et al.*, 1994). Thus, the leptin and leptin receptor are being intensively studied in these animals, with evidences for the therapeutic use of leptin in humans. However, it remains unclear whether the leptin-mediated control mechanism is conserved in non-mammalian vertebrates such as the avian. Several attempts to amplify the leptin gene in the chicken were made with no success (Friedman-Einat *et al*., 1999; Pitel *et al*., 2000; Amills *et al*., 2003, Ninov *et al*., 2008). Therefore, the leptin gene was not yet mapped in the chicken genome. In fact, there is even the possibility of no existence of the chicken leptin gene (Scanes, 2008). Considering the controversies regarding the existence of the leptin gene in chicken, the leptin receptor gene may provide insights on the leptin understanding. In

this study, the association of the LEPR A286G single nucleotide polymorphism (SNP) with carcass traits was investigated in the EMBRAPA TT Reference Population.

MATERIALS AND METHODS

The analyzed population was generated by the expansion of the paternal TT broiler line, developed by the EMBRAPA Poultry Breeding Program, to validate potential genetic markers and for gene discovery (Peixoto *et al.,* 2010). Data from 1412 chickens slaughtered at 42 days of age were used. A total of 32 carcass traits was evaluated, such as: carcass weight, weights of head, feet, neck, wings, middle joint wings, wing sticks, thigh, thigh muscle, breast, breast muscle, breast skin, breast fillet, thigh and drumstick muscle, back weight, drumstick yield, breast muscle yield, thigh and drumstick yield, and thigh and drumstick muscle yield.

Genomic DNA extraction from blood was performed with DNAzol (*Invitrogen*). Primers to amplify the region of interest in the LEPR were: Direct - 5'tctggagtgaatggagcaca3' and Reverse - 5'gctacgctctgggttttgtt3 (Ninov *et al*., 2007). The amplicon of 755 bp was obtained by the PCR reaction. The SNP LEPR1 A286G was identified within intron 6 of the chicken LEPR gene and was discriminated by PCR-RFLP using the *Hha* I restriction enzyme. Genotypes were classified in normal homozygotes (AA), heterozygotes (AG) and mutant homozygotes (GG) according to the DNA fragments size.

Association analyses were performed using QxPaK software (Perez-Enciso and Misztal, 2004) with a model including the fixed effects of sex, hatch and SNP, and the infinitesimal and residual random effects. The additive (*a*), additive + dominant (*ad*) and dominant (*d*) effects of the SNP were tested including their interaction with sex.

RESULTS AND DISCUSSION

The PCR-RFLP revealed the presence of 654 chickens with the homozygous genotype for the normal allele (AA), 680 heterozygous (AG) and 78 mutants homozygous (GG) for the SNP. In this study, the A286G SNP in the leptin receptor gene showed significant association with most of the carcass traits evaluated in broiler chickens (Table 1). The SNP effect was additive and the A allele had favorable effect for all traits with significant association, with exception for thigh and drumstick yield and thigh and drumstick muscle yield (%). For instance, the A286G SNP is associated (p= 0.0054) with carcass weight and the allele A has an additive favorable effect of 28.01 g. The LEPR A286G SNP was previously associated with performance traits in the same TT Population, with the A allele showing favorable effect for weight at 35, 41, 42 days, and body weight without feathers and blood (Peixoto *et al*., 2010).

Although the leptin and leptin receptor genes are well studied in cattle and pigs, there are inconclusive reports about leptin function in chicken. However, the leptin receptor gene was associated with a wide variety of traits of economic interest in poultry. The LEPR is located at approximately 29.1Mb of the chicken chromosome 8, in a region

where several QTLs have been reported, such as body weight, breast muscle weight, drumstick muscle weight, tibia weight, tibia width, etc. (QTLdb; http://www.animalgenome.org/QTLdb/). Wang *et al*. (2004) correlated LEPR polymorphisms with abdominal fat and liver weight in a population divergently selected for fat deposition. Ninov *et al.* (2008) found an association between the LEPR SNP C352T and carcass yield, breast yield, crude protein and ash content, only in males in a segregating population.

Table 1. The A286G LEPR SNP additive effect (a) and its standard error (se) for significant associated (p-value) carcass traits in a paternal broiler line.

Trait	**p-value**	**a (se)**
Carcass weight (g)	0.54E-03	28.01(7.99)
Head weight (g)	0.28E-04	0.67 (0.26)
Feet weight (g)	0.41E-02	1.12 (0.36)
Neck weight (g)	0.44E-02	2,49 (0,87)
Wings weight (g)	0.20E-04	3,23 (0,75)
Middle joint wings weight (g)	0.27E-03	1,10 (0,30)
Wing sticks weight (g)	0.41E-03	1.69 (0.48)
Thigh weight (g)	0.11E-01	2,75 (1,07)
Thigh muscle weight (g)	0.12E-01	2,75 (1,08)
Breast weight (g)	0.48E-04	11,46 (2,77)
Breast muscle weight (g)	0.18E-04	8,19 (1,88)
Breast skin weight (g)	0.43E-01	0,66 (0,33)
Breast fillet weight (g)	0.32E-03	2,00 (0,55)
Back weight (g)	0.19E-01	3,52 (1,50)
Drumstick yield (%)	0.28E-01	0,10 (0,05)
Breast muscle yield (%)	0.11E-01	0,13 (0,05)
Thigh and drumstick yield (%)	0.95E-02	-0,14 (0,05)
Thigh and drumstick muscle yield (%)	0.72E-02	-0,14 (0,05)

In this study, the results indicate that the LEPR SNP is associated to a set of carcass traits in chickens. The LEPR effect may be caused directly by the A/G exchange. It is known that even intron regions, that are not expressed, may be important for the regulation of gene expression. Another possibility is that the effect observed is due to linkage disequilibrium between the A286G SNP and other SNP which could be the causative mutation. In any case, this SNP can be considered as a potential genetic marker, due to the significant associations found. However, the consistent associations observed in a pure line, together with the LEPR gene location in the genome and its important biological function in mammals, led to the evidences that this gene might be directly responsible for the significant associations observed. The fact that the leptin receptor gene has a consistent effect in several growth, carcass and bone traits is quite interesting since the leptin gene in chickens has not been mapped yet. These findings

suggest a molecular evidence for the existence of a leptin-mediated control mechanism in poultry.

CONCLUSION

The results indicate that the A286G LEPR SNP is a potential genetic marker to improve carcass traits in chickens. These findings suggest a molecular evidence for the existence of a leptin-mediated control mechanism in poultry.

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