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New Insights on the Influence of Leptin Receptor Gene in Bone Traits in Broilers

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ABSTRACT: It is widely known that leptin receptor gene (*LEPR)* is related to adipose tissue storage and feed efficiency, being functional expressed in many tissues in chicken. In several species, this gene also plays essential role on growth and ossification. No information on the influence of *LEPR* in bone differentiation has been shown in chickens. Thus, we aimed to verify the association between bone integrity traits and the *LEPR* gene. Tibia and femur phenotypic data from 1,412 broilers with 42 days of age were collected. A SNP on the *LEPR* chicken gene was genotyped by PCR-RFLP, followed by association analysis using QxPaK software. Significant associations were found with femur zinc and tibia dry matter contents, tibia breaking strength, tibia weight, and femur length. The A allele was favorable to improve bone quality. This is the first report associating the *LEPR* with bone integrity in broilers.
Keywords: *LEPR*; chicken; SNP; ossification

Introduction

In order to improve the poultry production, companies and producers have favored the selection for heavier and faster growing broilers (Havenstein et al. (2003)). Meanwhile, leg disorders have increased exponentially, becoming one of the main causes of economic losses to the poultry industry. These negative effects are related to reduction of welfare, feed efficiency, growth traits and other factors (Cook (2000)). Therefore, selection against skeletal disorders should be prioritized. The elucidation of molecular pathways involved on growth and bone development might help the improvement in breeding genetics in order to produce animals with healthy bone traits while keep the production efficiency.

In many species, the leptin gene (*LEP*) is widely studied because of its function associated to adipose tissue storage and feed efficiency. Moreover, it has been suggested a role of the leptin hormone in endochondral ossification (Ornan et al. (2009)). However, in chicken, no insights about this gene is available, since many attempts to map the leptin gene were made with no success (Pitel et al. (2000); Amills et al. (2003); Ninov et al. (2008)). Despite of the leptin gene isolation issues in chicken, the leptin receptor gene (*LEPR)* is functionally expressed and may provide information about the leptin understanding, as well as, its importance on bone metabolism.

The influence of *LEPR* in carcass traits has been already reported in broilers, having an additive effect on breast, thigh and drumstick weights and yields (Peixoto et al. (2012)). Since limited information about the *LEPR* is provided in chicken, we aimed to evaluate the association of the A286G SNP in this candidate gene with bone integrity traits in a paternal broiler line.

Material and Methods

Animals. The analyzed population used in this study was provided by the expansion of the paternal TT broiler line, developed by the EMBRAPA Poultry Breeding Program. This line has been under multi-trait selection, with emphasis on body weight at 42 days of age (BW42). Chickens were kept in collective pens until 35 days of age and then housed in individual cages for feed conversion evaluation. Data from 1,412 chickens slaughtered at 42 days of age were used.

Genotyping. Genomic DNA extraction from total blood was performed with DNAzol® (Invitrogen) reagent. Primers to amplify the region of interest in the *LEPR* were: F - 5' TCTGGAGTGAATGGAGCACA 3' and R- 5' GCTACGCTCTGGGTTTTGTT 3' (Ninov et al. (2008)), producing a 755 bp amplicon. The SNP *LEPR* A286G, identified in the intron 6 of the *LEPR* chicken gene, was genotyped 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 observed in 2% agarose gel.

Bone integrity phenotypes. Bone integrity traits evaluated in the tibia and femur were: weight (g), length (mm), width (mm), dry matter content (%), ash content (%), calcium content (mg/Kg), phosphorus content (mg/Kg), magnesium content (mg/Kg), zinc content (mg/Kg), CA:P ratio, Seedor Index, flexibility (mm/Kgf), and breaking strength (Kgf). Samples were stored in freezer at -20 °C. Bone resistance analyses were performed in the texturometer TA – XTPlus Texture Analyzer ©Texture Technologies Corporation, using the 3-Point Bending Rig (HDP/3PB) with 5 kg load cell Heavy Duty Platform (HDP/90). The fragments generated after the breakdown were heated at 105 ºC for 20 hours to determine the dry matter. After this procedure, bones were burned in oven at 550 °C for 6 hours for ash content determination.

Statistical analyses. Association analyses were carried out 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) and the additive + dominant (ad) effects of the SNP were tested, including their interaction with sex.

Results and Discussion

Genotyping results showed that 654 chickens were homozygous for the normal allele (AA), 680 heterozygous (AG) and 78 mutants homozygous (GG). The A286G SNP in the leptin receptor gene had significant association with 5 out of the 24 bone traits evaluated in broiler chickens (Table 1). The A allele had a significant additive effect on femur zinc content (FZC), tibia dry matter content (TDMC), tibia breaking strength (TBS), tibia weight (TW), and femur length (FL). This A allele was associated to an increment for most of these bone integrity traits, except for TDMC.

Table 1. The A286G SNP additive effect on bone integrity traits

Traits	Mean ± SD	p-value	Effect±SD
FZC(mg/Kg)	374.44 ± 45.81	0.203E-01	6.20 ± 2.67
TDMC(%)	50.40 ± 3.95	0.615E-02	-0.61 ± 0.22
TBS(kgf)	31.47 ± 8.11	0.175E-03	1.68 ± 0.44
FL(mm)	69.55 ± 3.66	0.114E-03	0.50 ± 0.16
TW(g)	11.88 ± 2.25	0.372E-13	0.64 ± 0.07

FZC: Femur zinc content, TDMC: tibia dry matter content, TBS: tibia breaking strength, TW: tibia weight, FL: femur length.

These findings about the influence of *LEPR* on bone integrity traits are interesting because a wide variety of carcass traits has been already associated with this gene in the same population. For both performance (Peixoto et al. (2010)) and carcass traits (Peixoto et al. (2012)), the same A allele showed to be favorable to improve these characteristics, as it conferred better femur and tibia quality in this study. We showed that this gene has an additive effect on many traits that are commonly recognized to negative genetic correlation.

At the region of leptin receptor gene, located on chicken chromosome 8, some QTLs associated with tibia and femur has been already described (Sharmam (2007)). However, this is the first study that elucidates the association of *LEPR* with these traits in chickens. In humans and rodents, it was reported that this gene helps to increase bone mass due to an increased rate of bone formation. This receptor plays important role acting directly on osteoblast differentiation, as well as on the proliferation of chondrocytes (Ornan et al. (2009)). Probably, the *LEPR* has a similar conserved functionality in chicken as in other species, but it has not been already unraveled.

Since consistent associations were found with bone integrity traits, it is possible that *LEPR* controls important biological pathways in broilers other than the described previously, e.g. feed efficiency. Attempting to understand how *LEPR* mediates genes in different molecular pathways, especially in the ossification, we searched for putative microRNAs binding sites in this gene. We found that gga-miR-2128, gga-miR-449, gga-miR-34, gga-miR-1459, gga-miR-1458 and gga-miR-1571 microRNAs were predicted to act in the *LEPR* 3´UTR (http://www.targetscan.org/). One of them, the miR-34 has already been described to be involved with bone development genes in humans and mice (Singh et al. (2009)). In chicken, according to the prediction, this miRNA could regulate bone specific-genes beyond *LEPR,* which helps to explain the role of this receptor in the regulation and differentiation of bone cells. Therefore, further biological studies involving functional analysis are needed to establish the molecular function involved with the chicken *LEPR* gene and bone development.

Conclusion

The A286G *LEPR* SNP had a positive effect on femur zinc content, tibia breaking strength, tibia weight and femur length. Considering that the *LEPR* positively influences other important poultry production traits, this SNP can be a potential genetic marker to improve bone integrity without compromise broiler efficiency. Furthermore, these findings suggest molecular evidence that *LEPR* is functionally important in bone development and maintenance in poultry.

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