

336 **Seven SNPs in the Coding Sequence of Leptin Receptor Gene in Long-term Selected Japanese Quail Lines**

337 **Kemal Karabağ<sup>1\*</sup>, Sezai Alkan<sup>2</sup>, Taki Karlı<sup>3</sup>, Cengiz İkten<sup>4</sup>, İnci Şahin<sup>5</sup>, Murat Soner Balcıoğlu<sup>3</sup>,**

338 <sup>1</sup> Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, Turkey, 07058

339 <sup>2</sup>Department of Animal Science, Faculty of Agriculture, Ordu University, Turkey, 52200

340 <sup>3</sup> Department of Animal Science, Faculty of Agriculture, Akdeniz University, Turkey, 07058

341 <sup>4</sup> Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Turkey, 07058

342 <sup>5</sup> Department of Plant Protection, Faculty of Agriculture, Selçuk University, Turkey, 42030

343

344 **\*Correspondence**

345 Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, Türkiye, 07058

346 Tel: +90 242 3102506

347 Fax: +90 242 2274564

348 [karabag@akdeniz.edu.tr](mailto:karabag@akdeniz.edu.tr)

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350 **ABSTRACT**

351 The objective of this study was to identify SNPs in the coding sequence of the leptin receptor gene and to test for  
352 their possible association with 20 economically advantageous traits in 15 generations of 2 selected (HBW and  
353 LBW) and a control of Japanese quail. A 350-bp part of the leptin receptor coding region was amplified and  
354 sequenced and understood that the fragment contained 7 SNPs (GenBank: KP674322.1-KP674328.1) that were  
355 detected in 5 loci (T3216C, T3265C, T3265G, C3265G, T3303C, A3311G, and T3347C) in a total of 30  
356 individuals. The T3216C and T3303C SNPs located at the end of the codon were synonymous and did not affect  
357 the presence of proline. However, phenylalanine, leucine and valine were produced when the T3265C, T3265G  
358 and C3265G SNPs, respectively, were present. Glutamine or arginine was produced when the A3311G SNP was  
359 A or G, respectively, and serine was produced when the T3347C SNP was C. Although codons and amino acid  
360 sequences changed due to the second SNP, the secondary protein structure was not changed. However, the fourth  
361 and fifth SNPs changed both the amino acid sequences and secondary protein structure.  
362 Pairing the SNP loci with phenotypic traits created haplogroups. When all individuals were evaluated together,  
363 some of the differences between the haplogroups were statistically significant ( $p < 0.05$ ;  $p < 0.01$ ). These results  
364 showed that both the sequence and structure of the leptin receptor gene could be altered by long-term selection.  
365 However, to achieve a more precise understanding of the role of leptin, entire coding sequences of leptin and the  
366 leptin receptor should be studied.

367

368 **Keywords:** Leptin receptor, SNPs, Phenotypic traits, Selection, Japanese quail

369

## 370 INTRODUCTION

371 The control of feed intake, energy balance and fat deposition has high economic importance in farm  
372 animals. The accumulation of excess fat affects meat quality, fertility, productivity, and whole body metabolism  
373 (Macajova et al. 2004). Leptin activity at specific receptors in the hypothalamus suppresses feed intake, which  
374 increases the use of energy, and leptin is a polypeptide hormone that controls the body's energy balance (Taouis  
375 et al. 1998; Ashwell et al. 1999). Leptin stimulates liposis, insulin sensitivity, glucose utilization in muscle, and  
376 fatty acid oxidation in liver, muscle and adipose tissue (Schwartz et al. 1996; Ahima and Osei 2004).

377 The leptin gene was first cloned and sequenced from chicken in 1998 (Taouis et al. 1998; Ashwell et al.  
378 1999). DNA sequence analysis of leptin from several mammalian and avian species, including livestock animals,  
379 showed that leptin is highly conserved among vertebrate species. Among vertebrates, the leptin amino acid  
380 sequence shows approximately 80% homology (Doyon et al. 2001). Wang et al. (2014) sequenced the entire  
381 coding region of the leptin receptor in *C. coturnix japonica*. The presence of leptin in laying and broiler poultry  
382 genotypes decreases feed intake (Denbow et al. 2000; Kuo et al. 2005). Quail chicks that were given  
383 recombinant mouse leptin on the fifth embryonic day were removed from incubation earlier (5-24 hours) and  
384 reached a higher body weight than the control group (Lamosova et al. 2003). In male quails that were given  
385 leptin during the embryonic period, the testes were two-fold larger, and testosterone levels were higher compared  
386 to those of controls on the 35<sup>th</sup> day. Moreover, female individuals who were treated with leptin began laying  
387 eggs earlier, and the number of eggs from each individual was higher than that of the control group (Macajova et  
388 al. 2002).

389 Mutations in genes responsible for the biochemical activity of an organism, such as those in leptin, can  
390 result in quantitative differences. With advances in DNA sequencing methods and technologies, molecular  
391 studies in recent years, including those in livestock species, have focused on single nucleotide polymorphisms  
392 (SNPs). In parallel, determining the DNA sequences and SNPs of leptin and its receptors in relation to livestock  
393 yields has accelerated research efforts. Researchers are actively investigating the relationship between leptin and  
394 traits of high economic importance, such as feed intake (Lagonigro et al. 2003; Huang et al. 2011), fertility  
395 (Liefers et al. 2002), milk production (Banos et al. 2008; Chebel and Santos 2011) and meat production (Lusk  
396 2007; Boucher et al. 2011).

397 In this study, possibility of SNPs that might be occurred in long-term selection in the coding sequences of  
398 leptin receptor gene and their relationship with phenotypic traits were investigated in 15 generations of selected  
399 Japanese quail (*Coturnix coturnix japonica*).

400

## 401 MATERIALS AND METHODS

402 In this study, three different populations of Japanese quail were used as the research model. One  
403 population was used as the control (C), which was not selected previously. The other two populations were  
404 extremely different from each other because they were selected for 15 generations for high body weight (HBW)  
405 and low body weight (LBW) in two different previous projects (Project numbers 21.01.0121.30 and  
406 2003.03.121.004) that were performed at the Scientific Research Projects Coordination Unit of Akdeniz  
407 University.

408

409 *Collection of Eggs and Chick Rearing*

410 Fertilized eggs were collected from the HBW, LBW and C populations for a week and stored at 15-20 °C  
411 and 75-80% humidity. These eggs were incubated at 36.5 °C and 65% humidity for the first 14 days and at 36.0  
412 °C and 55% humidity for the last 4 days. Chicks were weighed individually using 0.01 g precision scales, and an  
413 aluminum ID number was attached to the left wings of chicks after incubation. These chicks (40 birds) were fed  
414 24% crude protein and 2900 kcal/kg ME (metabolic energy) during the first four weeks in a breeding cage. Sex  
415 determination was performed by observing the cloaca and breast feather color at the end of the fourth week.  
416 Males and females (n=50 for both) were selected randomly from each population and transferred to individual  
417 breeding cages for 10 weeks. All birds were fed 21% crude protein and 2800 kcal/kg ME for ten weeks. Lighting  
418 was applied continuously for the first four weeks and then 16 hours a day.

419

#### 420 *Body Weight and Feed Intake*

421 Body weights (B1W) were measured for each animal using 0.01 g precision scales from hatching (HIW)  
422 to the 15<sup>th</sup> week on the same day each week. The differences arising from feed intake (FI) were fixed because  
423 quails were not fed on the day of weighing. Thus, 10 weeks of individual feed intake (FI) were recorded as  
424 grams per day.

425

#### 426 *Sexual Maturity*

427 The first ovulation day and the first day of release foam were considered as sexual maturity for females  
428 and males, respectively. Females and males were weighed on the same day. In this manner, sexual maturity age  
429 (SMA) and sexual maturity weight (SMW) were determined.

430

#### 431 *Egg Yields of Females*

432 Egg yields (number and weight) of female quails that were transferred from individual breeding cages  
433 were recorded daily for 10 weeks. The total egg weight (TEW) and total egg number (TEN) were obtained.

434

#### 435 *Carcass Traits*

436 A total of 30 quails selected randomly as 5 females and 5 males from the HBW, LBW and C populations  
437 were introduced to the cutting process after five weeks of growth and yield during a ten-week period. The body  
438 weights of the selected animals were measured just before cutting. Low-voltage electrical current (100 mA, 50  
439 Hz) was used to stun animals as recommended in the relevant scientific literature (Göksoy et al. 1999), and then  
440 the jugular vein was cut. Separation of the carcasses of the slaughtered quails was performed according to the  
441 recommendations in the literature (Yalçın et al. 1995). The body weight (B1W), breast weight (B2W), back  
442 weight (B3W), carcass weight (CW), gizzard-null weight (GW), head weight (H2W), heart weight (H3W), liver  
443 weight (LW), left leg weight (LLW), left wing weight (LWW), right leg weight (RLW) and right wing weight  
444 (RWW) were measured using 0.01 g precision scales. The measurements of the carcass components were  
445 obtained immediately after cutting. Tibia bone length (TBL) and tibia bone width (TBW) were measured using a  
446 digital compass after cleaning and separating the surrounding body tissue. Bone lengths were measured from the  
447 proximal to distal ends of the bones.

448

#### 449 *Tissue Samples and Total RNA Extraction*

450 Liver tissue samples from each individual were isolated using sterile forceps and scissors immediately  
451 after cutting and placed into numbered tubes (Corning, New York, U.S.A.) containing RNAsave. These tissues  
452 were stored at -80 °C until use. Cellular degradation of the liver tissues was performed using a lyser with  
453 tungsten beads. Then, total RNA extraction was performed using a commercial kit (Axygen). The total RNA  
454 obtained from 30 quails was stored at -80 °C until use.

455

#### 456 *cDNA Synthesis and PCR Amplification*

457 A commercial kit (Thermo Scientific #K1621) was used to synthesize cDNA from total RNA using the  
458 following protocol: 60 min at 42 °C, 5 min at 70 °C. Primers (forward, gcttgctcaggtagctcctg and reverse,  
459 tgcggcagctatggcagcat) based on the recommendations of Dridi et al. (2005) were used to PCR amplify a 350 bp  
460 leptin receptor coding region from cDNA. PCR products (15 µl) were evaluated for a 350 bp length using 2%  
461 agarose gels (electrophoresed at 80 V/2 h) and stained with ethidium bromide. Separated fragments in the  
462 electrophoresis gel were cut with a sterile scalpel under UV light and transferred to individual 1.5 ml pre-  
463 numbered tubes. The PCR reactions were performed in 20 ml reaction volumes with 2 µl of genomic DNA (20  
464 ng) as a template, 2 µl of buffer (NH<sub>2</sub>SO<sub>4</sub>), 0.4 µl of a dNTP mix (2.5 mmol/L), 0.5 ml of each primer (20  
465 nmol/ml), 1.25 µl of MgCl<sub>2</sub> (25 mM) and 0.15 µl of EX Taq polymerase (Takara Bio Inc. Shiga, Japan).  
466 Amplifications were performed using a thermal cycler (Thermo Arktik) with the following conditions: 3 min for  
467 an initial denaturation at 94 °C, 35 cycles at 94 °C for 30 s for denaturation, 30 s for annealing at 60 or 62 °C, 45  
468 s for extension at 72 °C, and a final extension for 5 min at 72 °C. *β-actin* gene primers (forward,  
469 caaggagaagctgtgctactgtc and reverse, ttaatcctgagtcaagcgc) were used to determine that the PCR protocol  
470 worked.

471

#### 472 *Sequence Analysis, SNP Determination and Haplogroup Generation*

473 DNA samples were concentrated in the PCR and sequenced directly in a sequencing instrument  
474 (Beckman 8800) after being purified from a gel and denatured at 94 °C. A leptin receptor gene fragment of 350  
475 bp was sequenced for 30 individuals from the HBW, LBW and C genotypes. These sequences were compared  
476 with each other for the presence of SNPs. Seven SNPs were found in five loci. Haplogroups were created by  
477 pairing the SNP locus and phenotypic traits and formed by grouping SNPs both between and within genotypes.

478

#### 479 *Statistical Analysis*

480 To determine the location of the DNA fragment (350 bp) in the leptin receptor gene, the amino acid  
481 sequence of the DNA fragment and the secondary protein structures from these amino acid sequences were  
482 determined using the online software at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, <http://translate-protein.com/> and  
483 <http://www.biogem.org/>. The predicted amino acid changes were located, and the significant differences were  
484 investigated.

485

486 All phenotypic traits (B1W, B2W, B3W, H1W, H2W, H3W, RWW, LWW, RLW, LLW, LW, CW, GW,  
487 SMW, TEW, SMA, TEN, FI, TBW and TBL) were evaluated using a Kolmogorov-Smirnov test for the normal  
488 distribution of all the data from three groups together and each group separately. A Box-Cox transformation was  
489 applied to the trait data that was not normally distributed, and the converted data were tested again for normality.  
B1W, LW, SMW, TBL and TBWA showed a normal distribution, whereas other features did not. When the

490 normality test was applied to genotypes separately, several traits were not normally distributed, including FI,  
 491 GW, H1W, LW, LLW, LWW, RLW, SMA, TBW in LBW; CW, GW, H3W, SMA, TBL in HBW; GW, H1W,  
 492 H3W, SMA in control.

493 A t-test and Mann-Whitney U test were conducted for binary SNPs (3216, 3303, 3311, and 3347) and  
 494 haplogroups, respectively, and for traits that showed either a normal distribution or a non-normal distribution,  
 495 respectively, in individuals who clustered in the same population but with different SNP haplogroups. ANOVA  
 496 and Kruskal Wallis tests were applied for normally distributed data and non-normally distributed data,  
 497 respectively, which had three alternative haplogroups (3265). In addition, the  $\chi^2$  test statistic was used on each of  
 498 the SNPs to determine whether the ratio was due to genotype possession.

499 *One-way ANOVA Procedure*

500 *Model:*  $y_{ij} = \mu + g_i + e_{ij}$

501 *Where;*

502 *y:* traits; *g<sub>i</sub>:* i. SNP effect; and *e<sub>ij</sub>:* error.

503

504 **RESULTS AND DISCUSSION**

505 A 350-bp fragment of the leptin receptor gene was sequenced in 30 individuals from three genotypes  
 506 (Table 1). These fragments were BLAST searched against GenBank to confirm their identity as fragments of the  
 507 leptin receptor gene (*Coturnix coturnix japonica*; KJ639903.1). A 116 amino acid sequence is predicted for the  
 508 350-bp fragment (Table 1). The fragment was compared among 30 individuals, and seven SNPs were identified  
 509 at five loci (Table 2). The sequences and SNPs were published in the GenBank website with the accession codes  
 510 KP674322.1-KP674328.1 (<http://www.ncbi.nlm.nih.gov/nucleotide/>).

511

512 **Table 1.** A 350 bp part of the leptin receptor gene and its amino acid sequence are shown.

<b>A</b>	<b>C</b>	<b>S</b>	<b>G</b>	<b>S</b>	<b>S</b>	<b>W</b>	<b>E</b>	<b>L</b>	<b>G</b>	<b>S</b>	<b>E</b>	<b>A</b>	<b>F</b>	<b>L</b>
gct	tgc	tca	ggt	agc	tcc	tgg	gag	ctg	ggg	agc	gag	gca	ttc	ctc
<b>L</b>	<b>L</b>	<b>P</b>	<b>D</b>	<b>Q</b>	<b>P</b>	<b>D</b>	<b>S</b>	<b>R</b>	<b>P</b>	<b>C</b>	<b>R</b>	<b>T</b>	<b>L</b>	<b>I</b>
ctg	ctg	ccc	gac	cag	cct	gac	agc	cgg	ccc	tgc	agg	acc	ctt	ata
<b>F</b>	<b>S</b>	<b>E</b>	<b>G</b>	<b>F</b>	<b>S</b>	<b>E</b>	<b>P</b>	<b>S</b>	<b>E</b>	<b>Q</b>	<b>D</b>	<b>G</b>	<b>A</b>	<b>F</b>
ttt	tca	gag	gga	ttt	tca	gag	cct	tca	gag	cag	gat	ggt	gct	ttc
<b>T</b>	<b>A</b>	<b>G</b>	<b>G</b>	<b>Q</b>	<b>E</b>	<b>R</b>	<b>G</b>	<b>L</b>	<b>C</b>	<b>Y</b>	<b>L</b>	<b>G</b>	<b>M</b>	<b>T</b>
aca	gcc	gga	ggt	cag	gag	cga	ggt	ctc	tgt	tac	ctg	ggg	atg	aca
<b>S</b>	<b>L</b>	<b>G</b>	<b>K</b>	<b>R</b>	<b>E</b>	<b>N</b>	<b>G</b>	<b>I</b>	<b>F</b>	<b>L</b>	<b>T</b>	<b>Q</b>	<b>S</b>	<b>S</b>
tca	ttg	ggc	aaa	aga	gaa	aat	ggc	att	ttt	tta	aca	cag	agc	tcc
<b>R</b>	<b>L</b>	<b>R</b>	<b>C</b>	<b>H</b>	<b>F</b>	<b>H</b>	<b>T</b>	<b>A</b>	<b>D</b>	<b>L</b>	<b>L</b>	<b>R</b>	<b>G</b>	<b>V</b>
aga	ctg	agg	tgc	cat	ttc	cat	aca	gct	gat	cta	ctc	aga	ggt	gtg
<b>G</b>	<b>F</b>	<b>L</b>	<b>Q</b>	<b>D</b>	<b>T</b>	<b>P</b>	<b>P</b>	<b>N</b>	<b>L</b>	<b>N</b>	<b>A</b>	<b>F</b>	<b>I</b>	<b>Q</b>
ggg	ttt	ctt	cag	gat	aca	cct	cct	aat	tta	aat	gca	ttt	atc	cag
<b>S</b>	<b>S</b>	<b>I</b>	<b>K</b>	<b>A</b>	<b>I</b>	<b>V</b>	<b>P</b>	<b>Y</b>	<b>M</b>	<b>P</b>				
agc	agc	att	aaa	gcc	atc	gtg	cca	tac	atg	ccg	ca			

513 Upper rows are amino acids, bottom rows are codons

514 Seven DNA sequences with seven SNP loci were uploaded to GenBank  
 515 (<http://www.ncbi.nlm.nih.gov/nucleotide/KP674322>) under the accession numbers KP674322-328. Wang et al.  
 516 2014 uploaded the sequence of the entire leptin receptor mRNA (3579 bp) of *Coturnix coturnix japonica* to

517 GenBank under the accession number KJ639903.1 (<http://www.ncbi.nlm.nih.gov/nuccore/KJ639903.1>). The 350  
518 bp part of the sequence that we identified exactly matched bases from 3163 to 3513 of the 3579 bp leptin  
519 receptor sequence.

520

521 **Table 2.** The SNPs identified in 30 individuals of the C, HBW and LBW genotypes.

Genotypes	SNP locus	individuals									
		1	2	3	4	5	6	7	8	9	10
C	3216	t	c	t	t	c	c	c	c	c	t
	3265	t	t	t	t	t	t	t	t	t	c
	3303	c	c	t	t	c	c	c	c	c	t
	3311	a	a	g	g	a	a	a	a	a	g
	3347	t	t	t	t	t	t	t	t	t	t
HBW	3216	c	t	c	t	t	c	t	c	t	t
	3265	g	g	g	g	g	g	g	g	g	g
	3303	c	t	c	t	t	c	t	c	t	t
	3311	g	g	a	g	g	a	g	a	g	g
	3347	c	t	c	t	t	t	t	t	t	t
LBW	3216	c	c	c	c	c	c	c	c	c	c
	3265	c	c	t	c	c	c	c	t	c	t
	3303	t	c	c	c	c	t	t	c	c	c
	3311	g	g	a	g	g	g	g	a	a	a
	3347	t	t	t	t	t	t	t	t	t	t

522 C: control, HBW: High Body Weight, LBW: Low Body Weight

523 The codons and amino acid sequence of the leptin receptor were determined using the online program  
524 <http://translate-protein.com> (Table 1.). Other possible codons and amino acid sequences were determined for  
525 other SNPs of this DNA sequence. The other possible codons and amino acid sequences that were derived from  
526 these SNP loci were different from each other. Changes in the secondary protein structures due to the SNPs were  
527 determined using an online program (<http://www.biogem.org>).

528 There was no amino acid difference when the first and third SNP were C and T because these SNPs only  
529 changed the last nucleotide of the codon. However, phenylalanine (F), leucine (L) and valine (V) were formed  
530 when the second SNP was T, C and G, respectively. Glutamine (Q) and arginine (R) were formed when the  
531 fourth SNP was A and G, respectively. Leucine (L) and serine (S) were formed when fifth SNP was T and C,  
532 respectively.

533 Structural changes in the amino acid sequence and protein were examined to evaluate the functional  
534 effects of the SNPs. From the 350-bp fragment, 116 amino acids were formed. The secondary protein structure  
535 of leptin receptor according to the basic amino acid sequence showed 63.8%  $\alpha$ -helices, 59.5%  $\beta$ -sheets, 17.2%  
536 turns and 14.7% coils. The amino acid sequence was not altered because the first and third SNPs were found in  
537 the third nucleotide of the codons in question and thus resulted in no changes to the protein structure. The seven  
538 possible amino acid sequences were published in GenBank (<http://www.ncbi.nlm.nih.gov/protein/>) under the  
539 accession codes AKM16812.1-AKM16818.1.

540 Although codons and amino acid sequences changed due to the second SNP, the secondary protein  
541 structure was not changed. However, the fourth and fifth SNPs changed both the amino acid sequences and  
542 secondary protein structure. When the fourth SNP occurred, there were 3.8%  $\alpha$ -helices, 53.4%  $\beta$ -sheets, 17.2%

543 turns, and 19.0% coils, and for the fifth SNP, there were 62.9%  $\alpha$ -helices, 57.8%  $\beta$ -sheets, 17.2% turns and  
544 17.2% coils.

545 When a t-test and Mann-Whitney U test were performed for traits that have a normal distribution (B1W,  
546 LW, SMW, TBL and TBW) and not-normal distribution (B2W, B3W, CW, FI, GW, H1W, H2W, H3W, LLW,  
547 LWW, RLW, RWW and SMA), respectively, for SNP 1, statistically significant differences ( $p>0.05$ ,  $p>0.01$ )  
548 were found between SNP haplogroups, with the exception of LW, FI and G (Tables 3, 5).

549

550 **Table 3.** A t-test of the SNP haplogroups for the normally distributed phenotypic values for all individuals  
551 ( $\bar{X}\pm SE$ ).

SNP loci	alleles	N	BW1	LW	SMW	TBL	TBW
3216	c	20	207.27 $\pm$ 17.78*	3.82 $\pm$ 0.45	188.70 $\pm$ 16.20*	47.39 $\pm$ 1.26**	2.21 $\pm$ 0.18**
	t	10	278.88 $\pm$ 16.99*	4.31 $\pm$ 0.50	257.46 $\pm$ 20.11*	52.83 $\pm$ 1.05*	2.99 $\pm$ 0.17*
3303	c	18	218.00 $\pm$ 18.53	3.99 $\pm$ 0.48	197.97 $\pm$ 17.00	48.11 $\pm$ 1.35	2.28 $\pm$ 0.19
	t	12	250.85 $\pm$ 22.60	3.96 $\pm$ 0.49	232.10 $\pm$ 23.16	50.85 $\pm$ 1.49	2.76 $\pm$ 0.21
3311	g	16	235.50 $\pm$ 22.43	3.61 $\pm$ 0.45	214.14 $\pm$ 21.55	49.15 $\pm$ 1.52	2.50 $\pm$ 0.23
	a	14	226.16 $\pm$ 18.00	4.41 $\pm$ 0.51	208.74 $\pm$ 17.53	49.26 $\pm$ 1.38	2.44 $\pm$ 0.17
3347	c	2	348.12 $\pm$ 18.48*	5.06 $\pm$ 0.46	297.36 $\pm$ 0.35	55.82 $\pm$ 2.12	3.34 $\pm$ 0.06
	t	28	222.78 $\pm$ 14.10*	3.90 $\pm$ 0.36	205.50 $\pm$ 14.17	48.73 $\pm$ 1.03	2.41 $\pm$ 0.15

552 \* and \*\* with different letters differ significantly at  $P<0.05$  and  $P<0.01$ , respectively.

553 BW1: Body Weight, LW: Liver Weight, SMW: Sexual Maturity Weight, TBL: Tibia Bone Length, TBW: Tibia Bone Weight,

554

555 **Table 4.** Mann-Whitney U test of SNP haplogroups for the phenotypic values from all individuals ( $\bar{X}\pm SE$ ).

Traits	SNP loci			
	3216 (C:T)	3303 (C:T)	3311 (A:G)	3347 (T:C)
BW2	55.55 $\pm$ 4.97*	58.35 $\pm$ 5.21	63.63 $\pm$ 6.18	96.67 $\pm$ 5.54*
	74.81 $\pm$ 4.93*	67.40 $\pm$ 6.24	60.07 $\pm$ 5.13	59.49 $\pm$ 3.88*
BW3	37.40 $\pm$ 4.07**	40.61 $\pm$ 4.24	45.14 $\pm$ 4.92	74.06 $\pm$ 1.17*
	55.77 $\pm$ 3.41**	47.90 $\pm$ 5.28	41.68 $\pm$ 4.50	41.34 $\pm$ 3.17*
CW	141.73 $\pm$ 13.14*	150.31 $\pm$ 13.68	168.44 $\pm$ 17.03	266.16 $\pm$ 12.27*
	200.00 $\pm$ 12.88*	177.43 $\pm$ 17.55	152.83 $\pm$ 13.21	153.66 $\pm$ 10.24*
FI	1598.97 $\pm$ 145.38	1625.93 $\pm$ 160.90	1866.30 $\pm$ 227.06	2059.91 $\pm$ 275.45
	2185 $\pm$ 302.31	2047.54 $\pm$ 266.16	1712.60 $\pm$ 181.62	1775.62 $\pm$ 155.37
GW	3.22 $\pm$ 0.24	3.31 $\pm$ 0.25	3.51 $\pm$ 0.32	4.75 $\pm$ 0.045
	4.03 $\pm$ 0.36	3.76 $\pm$ 0.35	3.47 $\pm$ 0.27	3.40 $\pm$ 0.21
HW1	7.56 $\pm$ 0.33**	7.88 $\pm$ 0.36	8.20 $\pm$ 0.46	9.73 $\pm$ 0.49
	9.28 $\pm$ 0.43**	8.52 $\pm$ 0.51	8.06 $\pm$ 0.38	8.02 $\pm$ 0.31
HW2	7.88 $\pm$ 0.48**	8.24 $\pm$ 0.49	9.06 $\pm$ 0.69	12.05 $\pm$ 1.33
	10.31 $\pm$ 0.60**	9.36 $\pm$ 0.75	8.26 $\pm$ 0.46	8.45 $\pm$ 0.41
HW3	1.63 $\pm$ 0.15*	1.69 $\pm$ 0.19	1.82 $\pm$ 0.16	2.63 $\pm$ 0.14
	2.14 $\pm$ 0.14*	1.96 $\pm$ 0.17	1.78 $\pm$ 0.17	1.74 $\pm$ 0.12
LLW	15.25 $\pm$ 1.48*	16.07 $\pm$ 1.55	18.08 $\pm$ 2.01	29.82 $\pm$ 2.81*
	20.97 $\pm$ 1.87*	18.78 $\pm$ 2.09	16.09 $\pm$ 1.41	16.25 $\pm$ 1.15*
LWW	4.61 $\pm$ 0.45*	4.85 $\pm$ 0.48	5.03 $\pm$ 0.50	7.92 $\pm$ 0.97*
	5.74 $\pm$ 0.45*	5.20 $\pm$ 0.50	4.95 $\pm$ 0.49	4.78 $\pm$ 0.33*
RLW	15.60 $\pm$ 1.59*	16.50 $\pm$ 1.67	18.44 $\pm$ 2.05	29.95 $\pm$ 3.66*
	21.46 $\pm$ 1.82*	19.13 $\pm$ 2.11	16.53 $\pm$ 1.58	16.66 $\pm$ 1.22*
RWW	4.53 $\pm$ 0.41*	4.74 $\pm$ 0.44	5.07 $\pm$ 0.48	7.31 $\pm$ 0.19
	5.94 $\pm$ 0.46*	5.39 $\pm$ 0.51	4.92 $\pm$ 0.47	4.84 $\pm$ 0.33
SMA	42.90 $\pm$ 0.77	42.67 $\pm$ 0.84	41.44 $\pm$ 0.45	

556  $40.90 \pm 0.46$   $41.58 \pm 0.60$   $43.14 \pm 1.05$   
 557 \* and \*\* with different letters differ significantly at  $P < 0.05$  and  $P < 0.01$ , respectively.  
 558 BW2: Breast Weight, BW3: Back Weight, CW: Carcass Weight, FI: Feed Intake, GW: Gissard-null Weight, HW1: Hatching Weight, HW2:  
 559 Head Weight, HW3: Heart Weight, LLW: Left Leg Weight, LWW: Left Wing Weight, RLW: Right Leg Weight, RWW: Right Wing  
 560 Weight, SMA: Sexual Maturity Age

561 Additionally, when an ANOVA and Kruskal-Wallis test were performed for traits that are normally  
 562 distributed (B1W, LW, SMW, TBL VE TBW) and not-normal distribution (B2W, B3W, CW, FI, GW, H1W,  
 563 H2W, H3W, LLW, LWW, RLW, RWW and SMA) in SNP 2, statistically significant differences ( $p > 0.05$ ) were  
 564 found between SNP haplogroups, with the exception of SMA (Tables 5, 7). However, when a t-test and a Mann-  
 565 Whitney U test were performed for traits that have a normal distribution (B1W, LW, SMW, TBL and TBW) and  
 566 non-normal distribution (B2W, B3W, CW, FI, GW, H1W, H2W, H3W, LLW, LWW, RLW, RWW and SMA)  
 567 for SNP 3 and SNP 4, there were no statistically significant differences. On the other hand, significant  
 568 differences were found for the normally distributed B1W trait in SNP 5 and the non-normally distributed B2W,  
 569 B3W, CW, LLW, LWW, and RLW traits ( $p > 0.05$ ). There was no difference between the haplogroups in terms of  
 570 studied phenotypic characteristics in the control vs. LBW genotypes. A statistically significant difference was  
 571 found between haplogroups H3W and CW in SNP 4 and SNP 5, respectively, and in the HBW genotype  
 572 ( $p < 0.05$ ).

573

574 **Table 5.** ANOVA test of SNP haplogroups that have three alleles at the 3,265 (SNP 2) locus for normal  
 575 distribution phenotypic values from all individuals ( $\bar{X} \pm SE$ ).

alleles	traits				
	BW1**	LW**	SMW**	TBL**	TBW**
c	150.57±12.10	2.47±0.42	138.30±12.85	43.42±1.11	1.73±0.21
t	206.43±11.38	4.34±0.59	185.45±10.77	47.63±0.79	2.26±0.15
g	325.25±8.96	4.77±0.49	301.69±10.91	55.72±0.59	3.33±0.08

576 \*\* with different letters differ significantly at  $P < 0.01$ .

577 BW1: Body Weight, LW: Liver Weight, SMW: Sexual Maturity Weight, TBL: Tibia Bone Length, TBW: Tibia Bone Weight

578

579 **Table 6.** Kruskal-Wallis test of SNP haplogroups that have three alleles at the 3,265 (SNP 2) locus for non-  
 580 normal distribution phenotypic values from all individuals ( $\bar{X} \pm SE$ ).

alleles	traits						
	BW2**	BW3**	CW**	FI**	GW**	HW1**	HW2**
c	39.78	25.73	101.77	1347.36	2.45	6.49	6.55
	±2.89	±2.14	±6.88	±241.44	±0.16	±0.21	±0.34
t	55.09	38.34	140.32	1555.67	3.13	7.74	8.11
	±3.25	±3.20	±8.22	±195.45	±0.22	±0.33	±0.42
g	87.97	63.98	233.68	2439.03	4.75	9.93	11.10
	±3.18	±3.23	±7.90	±205.70	±0.17	±0.23	±0.57
alleles	HW2**	HW3**	LLW**	LWW**	RLW**	RWW**	SMA
c	6.55	1.28	10.74	3.19	10.89	3.32	42.50
	±0.34	±0.12	±0.69	±0.19	±0.66	±0.21	±0.73
t	8.11	1.56	14.65	4.32	14.68	4.25	41.92
	±0.42	±0.12	±0.75	±0.18	±0.79	±0.20	±0.96
g	11.10	2.50	25.29	7.23	26.33	7.25	42.40
	±0.57	±0.12	±1.49	±0.42	±1.32	±0.32	±1.14

581 \*\* with different letters differ significantly at  $P < 0.01$ .

582 BW2: Breast Weight, BW3: Back Weight, CW: Carcass Weight, FI: Feed Intake, GW: Gizzard-null Weight, HW1: Hatching Weight, HW2:  
583 Head Weight, HW3: Heart Weight, LLW: Left Leg Weight, LWW: Left Wing Weight, RLW: Right Leg Weight, RWW: Right Wing  
584 Weight, SMA: Sexual Maturity Age  
585

586 However, no statistically significant differences in the SNP haplogroups from the phenotypic parameters  
587 of the non-normal distribution were found when the genotypes were tested separately.

588 Additionally, a  $\chi^2$  test was performed to determine whether the SNP ratio depended on genotypes,  
589 according to the calculations of SNPs 1 and 2. The results showed that the SNPs were due to the genotype of the  
590 SNP ratio ( $p < 0.05$ ).

591 All statistical analyses suggested that SNPs 1 and 2 altered the function of the leptin receptor. However,  
592 conclusively demonstrating this effect requires the identification of all of the SNPs in the entire sequence of the  
593 leptin receptor in quails.

594 Although leptin function is controversial in chickens (Sharp et al. 2008), leptin is an excellent candidate  
595 gene for livestock production, as it is associated with features of economic importance. Indeed, in recent years,  
596 leptin gene polymorphism studies of several single nucleotide polymorphisms (SNPs) have been identified in  
597 cows and pigs (Jiang and Gibson 1999; Buchanan et al. 2002; Liefers et al. 2002; Buchanan et al. 2003;  
598 Lagonigro et al. 2003; Lusk 2007). Several SNPs have been identified that are associated with important  
599 economic traits, such as milk yield, feed intake, adiposity, growth, and carcass quality. DeVuyst et al. (2008)  
600 found that both crossbred CT and TT beef cows wean significantly heavier beef calves than CC cows. There  
601 were several SNPs found in the porcine *LEP* and *LEPR* genes, suggesting that the SNPs lead to increased growth  
602 and fat (Perez-Montarelo et al. 2012). Buchanan et al. (2003) revealed a SNP in the leptin gene of dairy cattle.  
603 This polymorphism, in which the first nucleotide is a thymine instead of cytosine in the 25<sup>th</sup> codon, changed  
604 arginine to a cysteine. However, homozygous animals carrying the T allele show no difference compared to  
605 animals carrying the C allele in terms of milk fat and milk protein production and a daily milk yield of more than  
606 1.5 kg. In addition, homozygous T allele-bearing animals developed higher fatty carcasses than those with the C  
607 allele (Buchanan et al. 2002). Leptin gene polymorphisms are used as DNA markers in marker-assisted selection  
608 (MAS) for commercial farming.

609 Feed consumption, growth, development, energy metabolism and immune system functioning have a high  
610 economic importance in livestock. In this regard, there is a need for additional studies on genes that affect animal  
611 feed intake, the regulation of energy metabolism, yield and health. Leptin plays an important role in all of these  
612 mechanisms of economic importance in livestock. Therefore, studies of leptin will significantly contribute to  
613 animal nutrition, breeding and health.

614 The effects of long-term selection in reared quail genotypes on the leptin receptor depend on the presence  
615 of specific SNPs, and these SNPs result in significant changes in the secondary structure of proteins. However,  
616 to achieve a more accurate understanding of the role of leptin and its receptor, the DNA sequence of all of the  
617 SNP changes that benefit individuals and alter protein structure should be identified.

618

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