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Association of leptin receptor gene (LEPR) variants with carcass traits and meat quality in broiler chickens

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Abstract

The aim of the study was to determine the lepton receptor (*LEPR*) gene polymorphism and its association with carcass traits, basic chemical composition, and physicochemical properties of breast and leg muscles, as well as texture and microstructure characteristics of the pectoralis major muscle. The research material consisted of 101 carcasses of 42-day-old fast-growing commercial Ross 308 broiler chickens. The breast meat samples were used to identify two single-nucleotide polymorphisms (SNPs) in the *LEPR* gene. Next, the values of carcass traits and meat quality traits were determined depending on four diplotypes (CC/GA, CT/AA, CT/GA, and TT/AA) of the studied gene. The results of LEPR genotyping showed the presence of three genotypes (CC, TT, and CT) for SNP g. 352C>T and two genotypes (AA and GA) for SNP g.427 G>A. The analyzed *LEPR*gene polymorphisms had a significant effect on carcass weight, abdominal fat percentage, and WB shear force and cutting work of the pectoralis major muscle. Broiler chickens with the CT/AA genotype had the lowest carcass weight, the lowest WB shear force and cutting work of the pectoralis major muscle (the best tenderness), and the highest abdominal fat content in the eviscerated carcass with the neck. Opposite results for these traits were found in chickens with the TT/AA genotype. The results of these studies indicate the possibility of using both SNPs in the *LEPR* gene in breeding programs as selection markers for reducing carcass fatness or improving the tenderness of Ross 308 broiler chicken breast meat.

Introduction

In recent years, increasing attention has been given to the possibility of identifying and using the polymorphisms of genes responsible for carcass and meat fatness in poultry. The genes responsible for shaping the fatty characteristics of the carcass include genes encoding proteins that participate in lipid metabolism and transport (including the fatty acid-binding protein *FABP* and heart-type fatty acid binding protein *HFABP* genes), genes controlling food intake and energy homeostasis of the organism (including ghrelin *GHRL*, melanocortin 3 receptor *MC3R*, and melanocortin 4 receptor *MC4R*). Additionally, adipocytokine genes that encode the production and secretion by adipose tissue (including leptin *LEP* and leptin receptor *LEPR*), or genes encoding proteins related to cell growth and development and skeletal muscle development (e.g., growth hormone *GH*, myogenic factor 5 *MYF5*, and myogenic factor 6 *MYF6*)[1].

The chicken cDNA *LEPR* was first identified more than 20 years ago[2,3]. Studies by Adachi et al.[4] and Hen et al.[5] demonstrated that the *LEPR* gene plays a crucial role in regulating body energy storage and metabolism. Huang et al.[6] also suggest that *LEPR* may influence lipid metabolism in chickens.

Research results show that the *LEPR* gene could be used to detect polymorphisms associated with growth and feed efficiency[7], carcass composition, including fat content[8-13]. El Moujahid et al.[7] indicate that four single-nucleotide polymorphisms (SNPs) (rs4657336, rs13684613, rs1368416, and rs13684616) are significantly associated with body weight (BW), feed intake (FI), and feed conversion ratio (FCR) in Chinese meat-type chickens. This study[7] also showed that the GTACGTSAC diplotype

resulted in the highest body weight gain (BWG) and feed intake (FI) in the N202 and 461 lines. Kaczor et al.[13] reported the association of LEPR gene polymorphism with BW, BW gain, slaughter value, as well as physicochemical characteristics and breast muscle tenderness in 42-day-old Ross 308 broiler chickens.

The present study aims to identify polymorphisms in the *LEPR* gene that are associated with desirable carcass and meat quality traits in broiler chickens. Specifically, it focuses on variants linked to reduced carcass fatness (lower content of skin with subcutaneous fat and abdominal fat), enhanced carcass muscling (greater breast and leg muscle yield), improved meat quality, and the optimal intramuscular fat content.

This article presents, for the first time, results concerning the influence of *LEPR* gene polymorphisms on microstructure and texture characteristics (except for WB shear force) and electrical conductivity of broiler chicken meat, which supplements the current state of knowledge in this field.

The study aimed to detect polymorphisms of the leptin receptor gene (*LEPR*) and to evaluate the effect of these polymorphisms on carcass weight and composition, basic chemical composition, physicochemical characteristics of breast and leg muscles, and texture and microstructure characteristics of the superficial breast muscle of 42-day-old Ross 308 broiler chickens.

Material and methods

Procurement and cutting of carcasses, sampling of meat for testing

The research material consisted of 101 eviscerated carcasses of 42-day-old Ross 308 broiler chickens. The carcasses were purchased from a small poultry slaughterhouse (Elczar, Serock near Bydgoszcz, Poland). The sex of the birds was identified during slaughter, after removing the eviscerated carcasses from the slaughter line (before cleaning), based on the appearance of the gonads. Fifty-five male and 46 female carcasses were purchased for the study. The eviscerated carcasses were transported to the university laboratory by car in a refrigerated box, and the transport time was 40 minutes. After delivery to the laboratory, the carcasses were immediately placed in a refrigerated cabinet (Hendi, Robakowo, Poland) and air-cooled for 24 hours at 4°C. After removing the carcasses from the refrigerator, the weight of each carcass was individually determined on an electronic scale (Radwag WLC 6/12/F1/R) with an accuracy of 0.1 g. Then, the acidity and electrical conductivity (pH_{24}, EC_{24}) of the pectoral superficialis and drumstick muscles were determined. Then, the entire gutted carcasses were dissected into parts according to the method described by Ziołecki and Doruchowski[1]. During dissection, the following parts were separated from each carcass: abdominal fat, skin with subcutaneous fat from the whole carcass, but without wing skin, two fillets and two tenderloins (pectoralis major and minor muscles on both sides of the breast), and all muscles of both thighs and drumsticks. The dissected carcass parts were weighed individually on an electronic scale (Radwag, WLC 6/12/F1/R), and then their percentage share in the eviscerated carcass with the neck was calculated. After dissection of the carcass, 101 samples of breast muscles and 101 samples of leg muscles were taken individually to

determine the basic chemical composition and color parameters of the meat, as well as 101 samples of m. pectoralis superficialis to determine the texture and microstructure characteristics, and 101 samples of m. pectoralis superficialis for genetic testing. The breast meat samples were used to identify two SNPs in the *LEPR* gene, and then the values of carcass traits and meat quality traits were determined depending on different diplotype variations of the tested gene.

Determination of basic chemical composition

The content of intramuscular fat, protein, collagen, and water in the breast muscles and leg muscles was determined using near-infrared (NIR) transmission spectrometry with Foss FoodScan™2 Meat Analyser according to PN-A-82109[15]

Evaluation of physicochemical traits

Before dissection, pH_{24} and EC_{24} measurements were taken of the *m. pectoralis superficialis* (breast muscles) and *biceps femoris* (drumstick) muscles (24 h postmortem). The pH of the meat was measured with a CX-701 pH meter (Elmetron, Zabrze, Poland). The pH meter was calibrated before measurement. Calibration buffers with pH 7.0 and pH 4.0 were used for this purpose. The electrical conductivity (EC_{24}) of the tested muscles - breast and drumstick meat - was measured using an LF-Star CPU (Ingenieurbüro R. Matthäus, Nobitz, Germany) conductivity meter. During the measurement, the device's electrodes were inserted into the breast or drumstick muscle at an angle of 90° along the muscle fiber orientation. The color coordinates *L** - lightness, *a** - relative redness, and *b** - relative yellowness[16] were marked on the inner surface - on the bone side - of the pectoralis superficialis muscle and leg muscles (thigh and drumstick muscles, after prior preparation and removal of the patella and tendons from both muscles) 24 hours postmortem. The measurements were performed using wide-area illumination (0° viewing angle, 8 mm – approximately 0.5 cm² measurement area, D65 illumination) using a ChromaMeter colorimeter (Konica Minolta, Chiyoda-ku, Japan) with a CR400 measuring device (head).

Determination of meat texture characteristics

The texture of muscle samples was assessed using the Texture Profile Analysis (TPA) method, as outlined by Bourne[17], with a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK). The analysis involved double compression of the sample along the orientation of the muscle fibers using a cylindrical probe with a diameter of 0.61 cm. Each compression was performed to 80% of the sample's original height (16 mm), at a crosshead speed of 50 mm/min, using a 50 N load cell. From the force-deformation curve obtained during the TPA test, the following texture parameters were calculated: hardness, cohesiveness, springiness, chewiness, and gumminess[17]. Each muscle sample was subjected to 7 replicate measurements.

Determination of meat structure characteristics

The average cross-sectional area (CSA) of muscle fibers, horizontal (H) and vertical (V) diameters, as well as the thickness of the endomysium and perimysium, were evaluated on raw samples taken from the central portion of the pectoralis major muscle. Two samples were collected from each bird. Tissue blocks measuring approximately 8 × 8 × 20 mm were prepared for microscopic examination following the method described by Burck[18] The samples were fixed in Sannomiya solution, dehydrated using alcohol and benzene, and then embedded in paraffin. The paraffin blocks were sectioned into 10 µm slices using a microtome. These tissue sections were mounted on glass slides, stained with hematoxylin and eosin, and sealed with Canada balsam[18].

The measurements of fiber CSA, diameters, and the thickness of endomysium and perimysium per muscle fiber bundle were conducted using the Multi Scan Base v.18.03 image analysis software [CSS Scan, Warsaw, Poland]. Six primary muscle fiber bundles were analysed for each muscle sample, under a microscope magnification of 100×.

Genetic studies

The samples of female (n = 46) and male (n = 55) broiler chickens were individually used for DNA extraction, which was performed using the High Pure PCR Template Preparation Kit (Roche Switzerland), according to the standard manufacturer's protocol. The concentrations and purity of the DNA isolates were assessed using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Germany). To check the quality of extracted DNA, samples were separated on 1.5% (p/v) agarose gel by electrophoresis. To identify SNPs of the *LEPR* gene, species-specific primers were used in PCR reactions. The primer sequences and restriction enzyme used in genotyping were as follows: F: ATGCTGCTTGATTCCTCC, R: CCCTAGGCAATGGTAATGAAC; PCR product size (bp): 502; restriction enzyme: Bsh1236I[13]

First, gradient PCR was used to determine the optimal annealing temperatures for the selected primers. PCR mix was prepared using GoTaq® G2 Flexi DNA Polymerase kit (Promega, Germany) comprising 5 µl of Green GoTaq® Flexi Buffer, 2.5 µl of $MgCl_2$ (25 mM solution), 0.5 µl of PCR Nucleotide Mix (10 mM), 0.125 µl of GoTaq® DNA Polymerase (5 u μl^{-1}), 0.5 µM of each primer, and 2 µl of DNA template, topped up with free-nuclease water to 25 µl. All amplifications were performed using T100™ Thermal Cycler (Bio-Rad, USA) and cycling conditions for gradient PCR were as follow: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at gradient of 50 – 65°C, and 30 seconds extension at 72°C; and a final extension at 72°C for 7 minutes. PCR products were evaluated by electrophoresis on a 1.5% agarose gel. Gradient PCR allowed for the selection of optimal annealing temperature for *LEPR* (61°C), and PCR cycling conditions were adjusted accordingly.

Two SNPs of the *LEPR* gene were assessed by digestion of 5 µl PCR product using 0.5 µl 10 U *Bsh*1236I enzyme,2 µl 10X Buffer R, topped up with 17.5 µl nuclease-free water (Thermo Fisher Scientific, Germany). Samples were mixed gently, incubated in a T100™ Thermal Cycler (Bio-Rad, USA) for 18 h at 37°C, and followed by enzyme inactivation at 65°C for 20 min. Restriction patterns for both genes were

visualised using a 2.5% agarose gel on GelDoc Go (Bio-Rad, USA). Next, genotypes were identified based on the following patterns (Figure 1), and genotype and allele frequencies were calculated (Table 1, 2)

Statistical analyses

For data concerning carcass composition, physicochemical properties, texture, and structure of the meat, arithmetic means and pooled standard error of the mean (SEM) were calculated for each trait. Normal distribution of the studied traits was analysed with the Shapiro-Wilk test. Analysis of variance was used to determine the effect of genotype on the analysed carcass and meat quality traits of the Ross 308 broiler chickens, including *LEPR* gene polymorphisms. The analysed carcass and meat quality traits were statistically analysed with SAS software ver. 9.4 - SAS Institute Inc.[19] Significant differences ($P \leq 0.05$) between the compared groups of broiler chickens will be determined by Tukey's test. During the genetic study, allele frequency was determined, and the association of the identified genotypes for each SNP with the analyzed traits of the broiler chickens was determined.

Ethical approval

Eviscerated broiler chicken carcasses and breast muscle samples were used for the study. The carcasses were purchased from a small poultry slaughterhouse (Elczar, Serock near Bydgoszcz, Poland). Before slaughter, the birds were kept on a commercial broiler farm under veterinary supervision as part of their agricultural production. Broiler chickens were reared in accordance with EU regulations (Directive 2007/43/EC) and the Polish Animal Welfare (Dz.U.2010/56/344), and their implementation did not require approval by the Local Ethics Committee for Animal Experiments.

Results and Discussion

Genotyping

The results of LEPR genotyping (Figure 1) showed the presence of three genotypes (CC, TT, and CT) for SNP g.352 C > T and two genotypes (AA and GA) for SNP g.427 G > A (Table 2). For the former SNP, the heterozygote CT was the most abundant (54.46%) in general, as well as in females (50.00%) and males (58.18%). The least frequent was homozygote TT (14.85%). Simultaneously, allele C was more abundant (57.92%) than allele T (42.08%). For the latter SNP, the heterozygote GA represented most of the sample genotypes (78.26% and 69.09% for females and males, respectively). The unchanged genotype GG was not detected among the analysed samples, while genotype AA abundance constituted 26.73% of all samples. Allele A was more frequent (63.37%) compared to allele G (36.63%).

Carcass characteristics

The effects of *LEPR* gene polymorphism on carcass weight (CW), abdominal fat (AF), skin with subcutaneous fat, which are indicators of carcass fatness, as well as pectoral muscle and leg muscle percentages, which are indicators of carcass muscularity, in 42-day-old fast-growing commercial Ross

308 broiler chickens are summarized in Table 3. Ross 308 broiler chickens with CT/GA and TT/AA genotypes had significantly ($P < 0.05$) higher CW than chickens with CC/GA and CT/AA genotypes. The highest carcass weight (2268.95 g) was found in broiler chickens with the TT/AA genotype, while the lowest CW was found in birds with the CT/AA genotype (2137.53 g). Concerning AF percentage, the significantly highest AF percentage was found in the carcasses of CT/AA chickens (1.02%). The AF percentage of CC/GA, CT/GA, and TT/AA chickens was lower by 0.24 percentage points, 0.28%, and 0.42%, respectively. This study also shows no significant ($P > 0.05$) differences between genotypes for skin with subcutaneous fat percentage, pectoral muscle percentage (PM), and leg muscle percentage (LM) in eviscerated carcasses with necks of 42-day-old Ross 308 broiler chickens. The highest skin with subcutaneous fat percentage (SF) was found in broiler carcasses with the TT/AA genotype, while the lowest SF was found in CT/AA broilers. The best musculature, total pectoral muscle, and leg muscle content was found in broiler chickens with the CT/AA genotype (50.62% of the carcass), while the lowest (49.46% of the carcass) was found in chickens with the TT/AA genotype. The differences obtained between genotypes in total PM and LM content and PM and LM content separately did not differ between genotypes. In studies by Kaczor et al.[13], a significant effect of *LEPR* gene polymorphism on BW, dressing percentage, and giblets percentage of fast-growing Hubbard Flex broiler chickens at 42 days of age was demonstrated. The association of *LEPR* gene polymorphism with breast, leg, and AF percentage in Hubbard Flex chickens[13] was not significant. In contrast, Wang et al.[20] show a significant effect of the *LEPR* gene on slaughter yield, leg muscle percentage, and heart percentage in Savimalt and French Giant meat-type quails. These authors[20] point to the possibility of using the *LEPR* gene as a molecular genetic marker in breeding work aimed at improving growth and carcass traits, as well as improving the economics of quail farming. The significantly higher percentage of FA in the carcasses of 42-day-old Ross 308 chickens with the CT/AA genotype in this study compared to CC/GA chickens CT/GA and TT/AA chickens in this study was probably related to the lower energy requirements of slower-growing CT/AA chickens and the storage of excess energy in the diet in the form of abdominal fat[21].

Basic chemical composition

The association of *LEPR* gene polymorphism with basic chemical composition of breast and leg muscles of Ross 308 broiler chickens at 42 days of age is presented in Table 4. The *LEPR* gene polymorphism had no significant effect on intramuscular fat, protein, collagen, and water contents in PM and LM. Ross 308 broiler chickens with the CT/AA genotype had lower intramuscular fat and collagen content and higher protein and water content compared to other genotypes (diplotypes). The *P* values for the basic chemical composition of BM ranged from 0.227 (intramuscular fat) to 0.701 (collagen percentage), while for LM, they were lower, ranging from 0.053 (intramuscular fat percentage) to 0.174 (water percentage).

Physicochemical meat traits

Statistical analysis of the association of *LEPR* gene polymorphism with pH_{24}, EC_{24}, color parameters - *L*, a*,* and *b** PM and LM in this study did not show significant differences between chickens with different

genotypes (Table 5). In contrast, Kaczor et al.[13] found a statistically significant effect of *LEPR* gene polymorphism on pH_{24} and *L** of *m. pectoralis major* fast-growing Hubbard Flex broiler chickens at 42 days of age. Broiler chickens with the CC/GG genotype had significantly higher pH_{24} of the breast muscle (pH_{24} = 5.83) compared to chickens with the CT/AA genotype (pH_{24} = 5.61). In the same study, Kaczor et al.[13] found significantly lower *L** values (darker color) in the PM of Hubbard Flex broiler chickens with the CC/GG genotype than in chickens with the CT/AA genotype.

Microstructure of meat

Analysis of statistical results on the association of *LEPR* gene polymorphism with microstructural characteristics of pectoral major muscle of Ross 308 broiler chickens at 42 days of age shows insignificant differences between genotypes for fiber cross-sectional area, fiber perimeter and diameters, as well as perimysium and endomysium thickness (Table 6). The highest values of fiber cross-sectional area, fiber perimeter, horizontal and vertical diameter, and perimysium thickness were found in broiler chickens with the TT/AA genotype, while the lowest values of these traits were found in chickens with the CT/AA genotype. In turn, endomysium thickness was highest in chickens with the CT/GA genotype, while the lowest values were found in chickens with the TT/AA genotype. Previous studies on *LEPR* gene polymorphisms in poultry indicate its association mainly with growth traits, feed utilization, and fat deposition, while there is no clear evidence of a direct link to muscle microstructure[9,20]. However, it can be assumed that the leptin–LEPR axis, by influencing muscle growth rate and energy metabolism, indirectly affects muscle fiber size and thus the remodeling of connective tissue within the muscle[22,23]. Thus, the presence of specific variants of the LEPR gene could promote changes in the organization of muscle microstructure, potentially affecting meat quality, including its tenderness and susceptibility to myopathy.

Texture of meat

Analysis of the results of the association of *LEPR* gene polymorphism with texture traits of the pectoral major muscle of Ross 308 broiler chickens aged 42 days evaluated in this study showed the effect of *LEPR* gene polymorphism on WB shear force and cutting work (Table 7). The best tenderness (lowest WB shear force) of the pectoral major muscle was found in chickens with the CT/AA genotype. Ross 308 broiler chickens with the TT/AA genotype had significantly higher WB shear force (worse tenderness) compared to chickens with the CC/GA, CT/AA, and CT/GA genotypes. The results of the measurements WB shear force *m. Pectoralis major* of broiler chickens with the TT/AA genotype was higher by 11.69%, 14.92%, and 9.90%, respectively. In addition, 42-day-old Ross 308 broiler chickens with the CT/AA genotype had significantly lower cutting work of the pectoralis major muscle compared to broiler chickens with other genotypes (Table 6). In the study by Kaczor et al.[13], no significant effect of *LEPR* gene polymorphism on breast meat tenderness determined by WB shear force values in 42-day-old commercial fast-growing Hubbard Flex broiler chickens was found. The WB shear force of the *pectoralis major muscle* of 42-day-old Hubbard Flex chickens[13] was lower (ranging from 13.64 to 14.99 N) than in

this study (ranging from 25.09 to 29.49 N, Table 7), which was probably related to the lower carcass weight and weaker musculature of Hubbard Flex chickens compared to the Ross 308 broiler chickens studied. As with microstructure, there is no clear evidence confirming a direct link between the *LEPR* gene in poultry and meat texture parameters. Studies comparing different genes regulating metabolism and growth have shown that polymorphisms in *LEPR* can affect meat pH and color, while shear force was significantly associated mainly with variants of other genes, including GHSR[13]. However, it can be assumed that by modulating the growth rate of muscle fibers, *LEPR* indirectly affects the mechanical properties of meat, which justifies the need for further research in this direction.

However, it seems that the histological structure of the muscle has a greater impact on the mechanical properties of meat. It has been found that larger muscle fibers are associated with increased shear force and greater hardness, which can worsen the tenderness of meat[22,24]. On the other hand, excessive thickness of the perimysium or endomysium, through an increase in the proportion of connective tissue in the muscle structure, leads to, among other things, increased chewiness and rubbery texture, while reducing the juiciness and desired tenderness of the final product[25,26]. However, no significant effect of endomysium thickness on the mechanical properties of meat was found. These relationships emphasize the importance of muscle microstructure in shaping the sensory characteristics of chicken meat and indicate the need to take it into account when assessing the quality of poultry raw material.

In conclusion, the analyzed *LEPR* gene polymorphisms only affected carcass weight, abdominal fat percentage, and the tenderness of the superficial breast muscle expressed by WB shear force and cutting work. Ross 308 broiler chickens aged 42 days with the CT/AA genotype had the lowest carcass weight, the lowest WB shear force and cutting work of the pectoral muscle (the best tenderness), and the highest abdominal fat content in the eviscerated carcass with the neck. Opposite results for these traits were found in chickens with the TT/AA genotype. Due to the limited number of scientific studies on the association of LEPR gene polymorphism with carcass traits and meat quality, it is necessary to continue research in this area in the future. Future studies should also be extended to include, among other things, consumer evaluation of sensory traits, determination of fatty acid profiles, and concentrations of selected minerals in broiler chicken meat in combination with *LEPR* gene polymorphisms.

Data availability

The datasets analysed during the current study are available from the corresponding author upon reasonable request. If there are some questions, the authors remain at your disposal.

Declarations

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Authorship contributions

Conceptualization, D.K., W.K., R.P; Investigation, D.K., W.K., N.Ś., R.P., P.E., J. Ż-K., M.K., and M.W.; Data curation, D.K.; Methodology, N.Ś., R.P., J. Ż-K., and M.K.; Writing – Orginal Draft Preparation, D.K., K.W., N.Ś., P.E., and J. Ż-K.; Writing – review & editing, D. K., K.W., N. Ś., R.P., P.E., J. Ż-K., M.W., and M.G. All authors have read and agreed to the publisher version of the manuscript.

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Competing interest

The authors declare no conflict of interest.

Additional information

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Tables

Table 1. Quantity and percentage of *LEPR* gene in samples of broiler chickens Ross 308.

Sex	Birds	Genotype (n)				Genotype (%)			
	(n)	CC/GA	CT/AA	CT/GA	TT/AA	CC/GA	CT/AA	CT/GA	TT/AA
Female	46	6	4	17	19	13.04	8.70	36.96	41.30
Male	55	9	8	14	24	16.36	14.55	25.40	43.64
Total	101	15	12	31	43	14.85	11.88	30.69	42.57

Table 2. Genotype and allele frequency of *LEPR* gene in female (n = 46) and male (n = 55) samples of broiler chickens Ross 308.

Sex	Genotype (%)					Allele (%)			
	CC	TT	CT	AA	GA	C	T	G	A
Female	36.96	13.04	50.00	21.74	78.26	61.96	38.04	39.13	60.87
Male	25.45	16.36	58.18	30.91	69.09	54.55	45.45	34.55	65.45
Total	30.69	14.85	54.46	26.73	73.27	57.92	42.08	36.63	63.37

Table 3 Association of LEPR gene polymorphism with carcass weight and some carcass elements of Ross 308 broiler chicken at 42 days of age. [a,b] Values within a row with different superscripts differ significantly between genotypes at $P < 0.05$. Genotype CC/GA, n = 16; CT/AA, n =12; CT/GA, n =31; TT/AA, n =43.

Trait	Genotype				SEM	P value
	CC/GA	CT/AA	CT/GA	TT/AA		
Carcass weight (g)	2142.68[b]	2137.53[b]	2264.13[a]	2268.95[a]	26.24	0.043
Abdominal fat (%)	0.78b	1.02a	0.74b	0.60b	0.03	<0.001
Skin with fat (%)	8.59	8.06	8.58	8.91	0.13	0.402
Pectoral muscle (%)	31.07	30.93	30.91	30.07	0.23	0.503
Leg muscle (%)	18.55	19.69	18.75	19.39	0.17	0.141

Table 4. Association of *LEPR* gene polymorphism with basic chemical composition of breast and leg muscle of Ross 308 broiler chicken at 42 days of age. Genotype

CC/GA, n = 16; CT/AA, n =12; CT/GA, n =31; TT/AA, n =43.

Trait		Genotype				SEM	*P* value
		CC/GA	CT/AA	CT/GA	TT/AA		
Intramuscular fat (%)	PM	1.53	1.52	1.96	1.93	0.09	0.227
	LM	5.93	6.35	6.65	7.03	0.15	0.053
Protein (%)	PM	23.67	23.78	23.51	23.52	0.06	0.384
	LM	19.69	19.57	19.38	19.22	0.07	0.102
Collagen (%)	PM	1.58	1.49	1.55	1.56	0.01	0.701
	LM	1.70	1.72	1.78	1.74	0.01	0.096
Water (%)	PM	72.79	73.23	72.66	72.69	0.08	0.323
	LM	71.48	71.27	71.15	70.86	0.11	0.174

Table 5. Association of *LEPR* gene polymorphism with some physicochemical properties of breast and leg muscle of Ross 308 at 42 days of age. Genotype

CC/GA, n = 16; CT/AA, n =12; CT/GA, n =31; TT/AA, n =43.

Trait		Genotype				SEM	*P* value
		CC/GA	T/AA	CT/GA	TT/AA		
pH_{24}	PM	6.57	6.54	6.57	6.60	0.02	0.673
	LM	6.52	6.48	6.51	6.48	0.02	0.924
EC_{24} (mS/cm)	PM	11.16	11.67	11.36	11.26	0.16	0.985
	LM	9.72	10.12	9.43	9.27	0.14	0.429
L* - lightness	PM	52.65	52.90	53.32	52.84	0.27	0.789
	LM	50.67	50.10	51.06	51.77	0.37	0.667
a* - redness	PM	2.44	2.77	2.48	2.97	0.12	0.589
	LM	5.34	5.48	6.02	6.46	0.28	0.580
b* - yellowness	PM	2.70	1.92	2.63	2.34	0.16	0.683
	LM	2.19	2.11	2.94	3.72	0.22	0.061

Table 6. Association of *LEPR* gene polymorphism with microstructural characteristics of pectoralis superficialis muscle of Ross 308 broiler chicken at 42 days of age.

Genotype CC/GA, n = 16; CT/AA, n =12; CT/GA, n =31; TT/AA, n =43.

Trait	Genotype				SEM	*P* value
	CC/GA	CT/AA	CT/GA	TT/AA		
Fiber cross-sectional area (μm^2)	1343.90	1304.84	1346.31	1378.40	22.78	0.460
Fiber perimeter (μm)	155.42	152.55	155.01	157.46	1.29	0.305
Fiber diameter H (μm)	43.32	42.38	42.41	43.42	0.46	0.245
Fiber diameter V (μm)	42.70	42.31	43.69	44.05	0.47	0.468
Perimysium thickness (μm)	19.76	19.17	19.49	20.71	0.46	0.686
Endomysium thickness (μm)	2.04	2.04	2.05	1.98	0.02	0.423

Table 7. Association of *LEPR* gene polymorphism with textural traits of pectoralis superficialis of Ross 308 broiler chicken at 42 days of age. a,b Values within a row with different superscripts differ significantly between genotypes at $P<0.05$. Genotype CC/GA, n = 16; CT/AA, n =12; CT/GA, n =31; TT/AA, n =43

Trait	Genotype				SEM	*P* value
	CC/GA	CT/AA	CT/GA	TT/AA		
WB shear force (N)	26.04^b	25.09^b	26.57^b	29.49^a	0.49	0.040
Cutting work (J)	0.22^a	0.19^b	0.21^a	0.23^a	0.01	0.027
Hardness (N)	15.65	15.38	15.34	16.64	0.18	0.145
Cohesiveness	0.34	0.34	0.34	0.33	0.01	0.831
Springiness	1.63	1.62	1.67	1.63	0.01	0.117
Chewiness (N)	8.71	8.40	8.69	9.22	0.15	0.499
Gumminess (N)	5.31	5.18	5.22	5.60	0.09	0.507

Figures

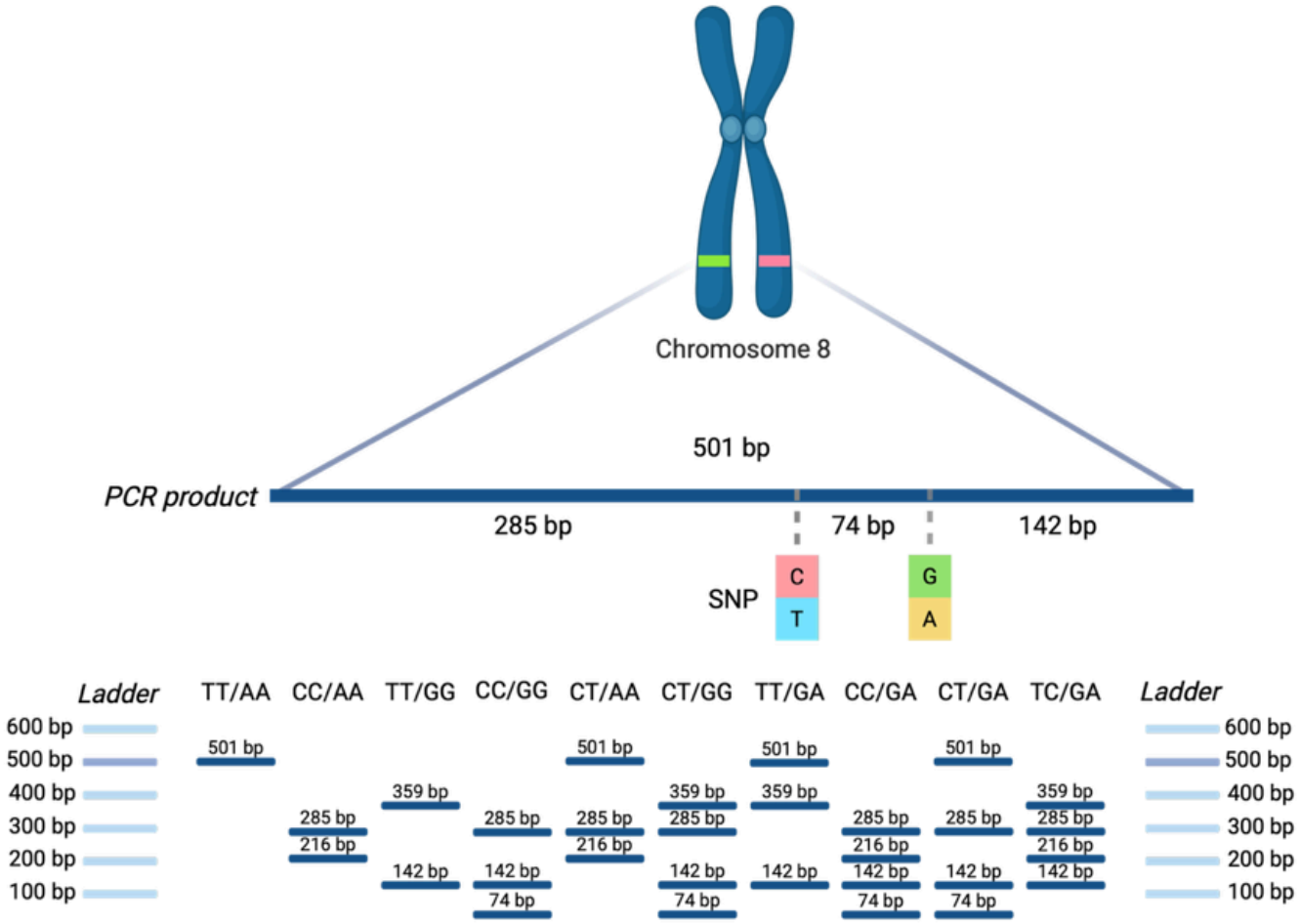


Figure 1

Detection of SNPs g.352 C > T and g.427 G > A in the *LEPR*gene using PCR-RFLP, pattern for digestion with Bsh1236I restriction enzyme, (based on Kaczor et al. 2016). Figure created in BioRender.

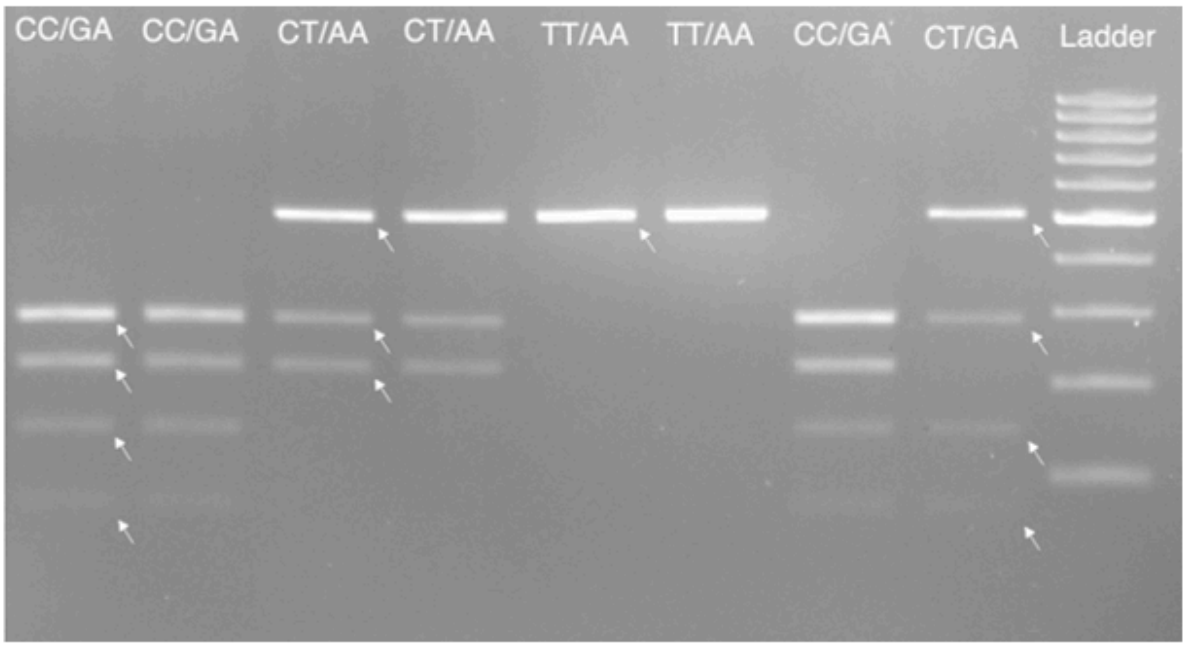


Figure 2

An example of *LEPR* gene fragment genotyping with *Bsh*1236I restriction enzyme. White arrows indicate digestion products identified on agarose gels. Ladder: 100-1000 bp.