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Polymorphism in the myostatin gene (*MSTN)* as related to growth and slaughter value of Ross 308 broilers* *

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The aim of the study was to identify single nucleotide polymorphisms (SNP) g.2242C>G and g.2373C>T at the myostatin gene (MSTN) locus in broiler chickens of the commercial Ross 308 line, reared to 45 days of age. No SNP g.2242C>G was found and the population was shown to be monomorphic. The g.2373C>T polymorphism was identified and the highest frequency was observed in cockerels of CT (0.43) and CC (0.36) genotypes. The T allele with substitution had a frequency of 0.43. The analysis made of cockerel growth until 45 days of age, slaughter value parameters and physicochemical traits of meat quality showed no effect of MSTN gene variation on the studied traits.

Key words: broiler chickens, slaughter value, meat quality, polymorphism, MSTN

Myostatin (MSTN, GDF8) protein is a member of the transforming growth factor-beta (TGF-beta) superfamily. GDF8 has a direct effect on the proliferation and differentiation of osteoprogenitor cells, and myostatin antagonists and inhibitors may enhance both muscle mass and bone strength (Elkasrawy and Hamrick, 2010). *MSTN* gene shows considerable variation in farm animals, and identification of the causes of double muscling in Belgian Blue cattle has attracted interest from researchers, resulting in the detection of many polymorphisms in subsequent years in many species, including cattle, sheep, pigs and horses (Han et al., 2013; Hill et al., 2010; McPherron and Lee, 1997; Qian et al., 2015). Research into variation in the hen *MSTN* gene was initiated by Baron et al. (2002), who identified its structure. The gene sequence of this species is similar to that in other vertebrate species. It is localized on chromosome 7 and contains 3 exons encoding 375–376 amino acids. It was found to contain several single nucleotide polymorphisms (SNP), including in Leghorn, local Chinese, Indian and Indonesian breeds (Mott Ivarie, 2002; Kumar et al., 2007; Khaerunnisa et al., 2016; Paswan et al., 2014). Ye et al. (2007) showed high variation of the DNA sequence in the *MSTN* gene and its association with body weight in Chinese broilers. c.234G>A SNP in exon 1 of the *MSTN* gene was identified in several flocks of Bian hens. The genotype was found to be associated with broiler growth between 6 and 18 weeks of age. These findings confirm that the myostatin gene mutation could be used as a genetic marker in poultry breeding (Zhang et al., 2012).

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Products of animal origin are the mainstay of global food production. The last five decades have witnessed meat production to increase by as much as 4 times (Kwasek, 2013). Broiler chickens are the basic slaughter material and account for as much as 86% of all birds slaughtered for meat internationally (Nowak and Trziszka, 2010).

Since the 1990s, breeding firms have offered several new commercial lines of broiler chickens with a particularly high proportion of breast muscles. Noteworthy among these is the Ross 308 line, which has been available since 2005, first as line 208, later as lines 308, 508 and 708. The Ross 308 line is one of the most popular lines of broiler chickens raised in Poland. It is characterized by very good growth rate, high resistance to disease, good survival, optimal feed conversion and good dressing percentage.

The objective of the study was to identify g.2373C>T and g.2242C>G polymorphisms in exon 1 of the myostatin gene in Ross 308 broilers and to determine the effect of variation on their growth and dressing percentage.

Material and methods

Material

The study material consisted of 141 Ross 308 cockerels. All chicks originated from a commercial hatchery in Łężkowice. One-day-old, individually tagged chicks were placed on deep litter in compartments (3.5 m^2) and reared under standard environmental conditions (temperature, relative humidity, light) at a stocking density of 33 kg/m^2. Throughout rearing, birds were fed the same complete diets *ad libitum*: starter (from 1 to 21 days), grower (from 22 to 35 days) and finisher (from 35 to 45 days of age), which contained, respectively, 22%, 20.5% and 20.5% crude protein and 2990, 3130 and 3130 kcal/kg. Birds had free access to water during the entire rearing period. Chickens were reared at the Experimental Poultry Farm of the National Research Institute of Animal Production in Aleksandrowice.

Methods

Evaluation of meat traits

During the rearing period, individual body weight of cockerels was recorded at one-week intervals and on day 45 of age. Prior to slaughter on day 45 of age, chickens were subjected to an 8-hour feed withdrawal. During slaughter, blood was colleted from the jugular vein to isolate genomic DNA. The carcasses obtained after post-slaughter processing were chilled for 24 h at 4°C and weighed. Chilled carcasses wee subjected to simplified slaughter analysis to determine the weights of breast and leg muscles, edible giblets (gizzard, liver and heart), leg bones, and abdominal fat. These data were used to calculate dressing percentage with and without giblets, and the percentages of breast and leg muscles, giblets, leg bones and abdominal fat in relation to the carcass weight with giblets.

Breast muscles (*pectoralis superficialis*) from all the broiler chickens under analysis were evaluated for selected technological parameters, including colour, acidity, thermal loss, and shear force. The colour of breast muscles was determined with the CIE L*a*b* scale 24 h postmortem using a Minolta CR 310 chroma meter (Minolta Camera C, Osaka, Japan). The inner surface of the left superficial pectoral muscle was measured immediately after it was removed from bones. Surfaces without discoloration, petechiae, visible blood vessels and meat defects were chosen for the analysis. Each sample was subjected to six measurements and the means were calculated for the colour coordinates L* (lightness), a* (redness), and b* (yellowness).

Muscle acidity was determined with a portable pH meter CyberScan10 (Eutech Cybernetics, Singapore) equipped with a glass electrode for meat analysis and a temperature probe for automatic temperature compensation. The measurement was made 24 h postmortem by placing the electrode at an 45° angle halfway through the left superficial pectoral muscle thickness. Before the analysis, the electrode was subjected to two-point calibration in calibration buffers pH 4.01 and 7.00, the temperatures of which were close to the measurement temperature.

Thermal loss was determined from the percentage cooking loss of breast muscles. Samples weighing around 190 g (e = 0.001 g) were placed in tightly closed plastic bags and cooked at 100°C for around 15 minutes until the internal temperature reached 76°C in the thickest point of the sample. After cooking, the samples were chilled at room temperature for 30 minutes and then at 4°C for 45 minutes. Thermal loss was calculated by subtracting the cooked sample weight from the uncooked sample weight, and then by dividing the obtained result by the uncooked muscle sample.

Shear force was determined by measuring the maximum force needed to cut the sample of cooked breast muscles. The analysis was performed using an Instron 5542 tensile tester (Instron, High Wycombe, UK) equipped with a Warner-Bratzler shear blade. Cylinders, 1.27 cm in diameter and around 3 cm in length, were cut from the thickest upper part of the cooked and chilled right superficial pectoral muscle. The so prepared sample was cut using a Warner-Bratzler blade with a triangular notch at three points, perpendicular to the muscle fibre orientation.

DNA isolation

Jugular blood was drawn from 45-day-old birds at slaughter and placed in EDTA anticoagulant tubes. Genomic DNA was isolated using the MasterPure Genomic DNA Purification Kit (Epicentre). The suitability of isolated DNA solution for further analyses was determined with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific) by analysing DNA purity and concentration in the solution.

PCR-RFLP

PCR was used to amplify DNA fragment of the *MSTN* gene. The primer sequence synthesized by Genomed (Warsaw) was taken from Ye et al. (2007). The primer had the following sequence:

(F) 5'-TAGTCAGCCCACAGAGAACG-3'

(R) 5'-CGAAAGCAGCAGGGTTGTTA- 3'

Primer annealing temperature was obtained by performing a temperature gradient in Eppendorf Mastercycler EP Gradient Thermal Cycler. Reaction mixture had a volume of 20 µl and contained 1x polymerase buffer, 2.5 mM of $MgCl_2$, 0.2 mM of each primer, 0.25 mM of dNTP, 1.2 of U polymerase (Thermo Fisher Scientific), 200 ng of genomic DNA. The thermal programme was as follows: 94°C – 3 min; 35 cycles: 94°C – 30 s, 59°C – 30 s, 72°C – 5 min.

To detect single nucleotide polymorphism in exon 1 of the myostatin gene, *Hin*P1 enzyme was used to identify g.2242C>G mutation, in addition to *Bbs*I enzyme (g.2373C>T mutation). The results of digestion with restriction enzymes were evaluated on 3% agarose gel with the marker O'GeneRuler Ultra Low Range DNA Ladder (Fermentas) at 80V and 400 mA for 40 minutes.

Statistical analysis

Allele frequencies and genotype frequencies were calculated by comparing the number of animals having a given genotype with the number of all animals. The $\chi 2$ test was used to check whether the analysed population is in Hardy-Weinberg's equilibrium. To determine the effect of genotype on growth parameters, slaughter value and meat quality traits, one-way analysis of variance was applied using the GLM procedure of STATISTICA ver. 13 (StatSoft Polska). For multiple comparisons, Tukey's test for unequal numbers was used.

Results

The amplification reaction produced a fragment of 321 bp, which was part of exon 1 of the *MSTN* gene (ID AF346599). No g.2242C>G substitution was identified in the analysed fragment, the entire population was monomorphic and all chickens had CC genotype.

The identification of g.2373C>T substitution using *Bps*I restriction enzyme showed the presence of C and T alleles and three possible genotypes (Table 1).

Table 1. Frequency of genotypes and alleles at the g.2373C>T locus in Ross 308 broilers

Genotype frequency			Allele frequency	
CC	CT	TT	C	T
0.36	0.43	0.21	0.57	0.43

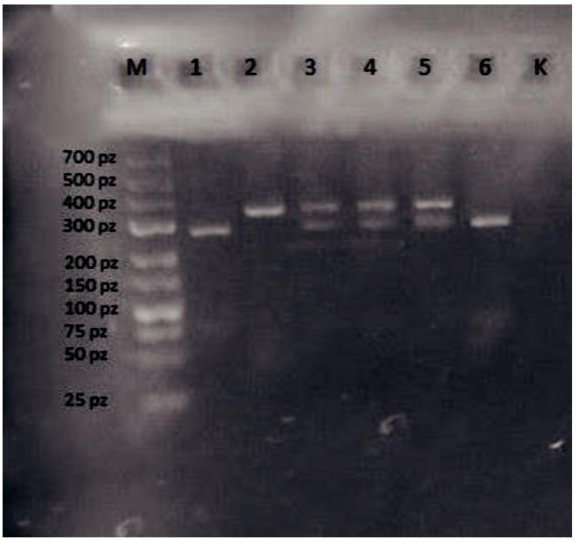

Fig. 1. *BpsI* enzyme digestion products (g.2373C>T) on agarose gel. M – size marker GeneRuler Low Range DNA Ladder, K – control sample, lanes 1 and 6 are TT genotype, lane 2 is CC. CT heterozygotes are in wells 3, 4 and 5, bands of 321 bp, 275 bp and 41 bp

The most frequent was allele C (0.57) and chickens of CT (0.43) and CC genotypes (0.36) (Fig. 1). The analysed population was in Hardy-Weinberg's equilibrium.

The anasysis of the relationship between g.2373C>T locus genotype and cockerel body weight, determined in successive weeks until birds reached the final body weight at 45 days of age, showed no statistically significant differences (Table 2). However, it should be noted that the highest initial and final body weight was exhibited by cockerels with TT genotype.

Table 2. Least square means (LSM ±SE) for body weight of chickens of different genotypes at the g.2373C>T locus in the *MSTN* gene

Body weight (g)	*MSTN* genotype		
	CC	CT	TT
day 1	45.27±0.61	45.65±0.63	46.42±0.84
day 7	162.55±2.48	166.42±2.68	153.88±3.16
day 14	466.25±7.40	460.81±6.65	441.92±9.34
day 21	988.25±13.27	969.38±12.67	945.00±18.11
day 28	1703.60±20.90	1651.00±19.19	1620.00±29.09
day 35	2414.00±27.76	2357.69±21.46	2367.69±35.47
day 45	3446.58±28.00	3529.86±27.55	3540.73±26.55

The effect of genotype was not significant for 14 post-slaughter parameters and 4 physicochemical traits of breast muscles (Tables 3 and 4).

Table 3. Means (LSM ±SE) for post-slaughter parameters of chickens of different genotypes at the g.2373C>T locus in the *MSTN* gene

Parameter	*MSTN* genotype		
	CC	CT	TT
1	2	3	4
Chilling loss (%)	1.70±0.33	1.73±0.54	1.71±0.26
Carcass weight after chilling (g)	2585.38±22.97	2651.65±21.20	2693.64±28.14
Dressing percentage with giblets (%)	77.99±0.23	78.09±026	78.60±0.26
Dressing percentage without giblets (%)	75.00±0.24	75.14±0.27	75.67±0.29
Breast muscles (g)	854.23±9.97	886.35±9.06	876.73±20.17
Breast muscles (%)	31.86±0.25	32.24±0.22	31.51±0.77

Table 3 – contd.

1	2	3	4
Leg muscles (g)	539.27±7.34	558.13±9.36	565.49±14.16
Leg muscles (%)	20.37±0.21	20.33±0.26	20.36±0.39
Giblets (%)	3.83±0.05	3.79±0.06	3.72±0.08
Liver (%)	2.46±0.05	2.45±0.05	2.34±0.06
Gizzard (%)	0.81±0.02	0.80±0.02	0.80±0.04
Heart (%)	0.57±0.01	0.54±0.01	0.58±0.03
Leg bones (%)	4.47±0.06	4.64±0.07	4.44±0.07
Abdominal fat (%)	1.35±0.05	1.13±0.05	1.28±0.12

Table 4. Least square means (LSM ±SE) for meat quality parameters of cockerels of different genotypes at the g.2373C>T locus in the *MSTN* gene

Parameter	*MSTN* genotype		
	CC	CT	TT
pH24	5.79±0.01	5.83±0.02	5.77±0.02
Colour:			
L*	59.08±0.40	58.54±0.40	59.12±0.50
a*	10.52±0.23	10.22±0.24	10.48±0.33
b*	9.17±0.20	9.46±0.20	9.89±0.28
	25.53±0.58	25.23±0.36	25.91±0.84
Thermal loss (%)			
	19.01±0.65	18.53±0.44	18.35±0.55
Shear force (N)			

Discussion

The identification of g.2242C>G and g.2373C>T polymorphisms at the *MSTN* gene locus in Ross 308 cockerels only showed the presence of g.2373C>T substitution. Allele T and CT heterozygotes with a frequency of 0.43 were identified. The present results are similar to the findings of Ye et al. (2007), where allele T frequency in three commercial lines of Chinese broilers (X, Y and Z) ranged from 0.17 to 0.52.

Genotype had no effect on the parameters of growth and dressing percentage. These results do not confirm the results of Ye et al. (2007), who found the same polymorphism (g.2373C>T) to have a positive effect on body weight gains of birds from 7 to 40 days of age. The presence of this polymorphism in the *MSTN* promoter was reported by Paswan et al. (2014) who identified three genotypes in chickens, with the most frequent being AA (63%) and AB (28%). The dominant allele was allele A with a frequency of 0.76. The authors observed that birds having allele A with a mutation exhibited higher body weight at 4, 5 and 6 weeks of age. On the day of hatch, chicks of BB genotype were characterized by considerably higher body weight compared to birds of the other genotypes (Paswan et al., 2014).

The analysis of LSM for postmortem traits of Ross 308 broilers showed that TT homozygotes had the highest values of parameters such as carcass weight after chilling, dressing percentage with and without giblets, weight of leg muscles, and weight of bones. CT heterozygotes were characterized by the greatest weight of breast muscles and a high fat content of these muscles. Wild-type CC homozygotes were characterized by the highest total content of giblets and abdominal fat, but at the same time achieved the lowest values for traits such as carcass weight after chilling, dressing percentage with and without giblets. However, all these differences were not significant. It has to be noted that most of the desirable traits decisive for production profitability and efficiency, such as carcass weight after chilling, dressing percentage with giblets, and the relatively low content of abdominal fat compared to others, were identified in TT homozygotes.

The present study also analysed the effect of polymorphism on meat quality parameters such as pH_{24}, meat colour, thermal loss, and shear force. Meat pH_{24} was similar in all the three genotypes. The meat colour parameters, such as L* (lightness) and b* (yellowness) had similar values for all the genotypes under analysis. Only the a* coordinate (redness) was lowest in CC homozygotes and highest in TT homozygotes. Thermal loss values were also similar in all the analysed chickens. Shear force value of meat from the cockerels with CC genotype was slightly higher than the value obtained for the other genotypes. Many authors analysed variation of the *MSTN* gene in local, Asian breeds and lines. The application of PCR-SSCP technique in three lines of Bian chickens revealed four SNP (G2283A, C7552T, C7638T and T7661A). The same study showed that chickens with mutations were characterized by higher body weight gain than birds without any mutations. The authors believed that G2283A mutation detected in exon 1 could become a genetic marker for breeders of Bian chickens (Zhang et al., 2011). In the same breed, Genxi et al. (2014) identified a number of SNP in 5' UTR and confirmed their relationship with growth parameters. In the 3'UTR region Zhiliang et al. (2004) detected g.7263A>T polymorphism and showed its correlation with the weight and percentage of breast muscles in the carcass. Khaerunnisa et al. (2016), who analysed the effect of g. 4842 T>G substitution in 7 Indonesian broiler populations, demonstrated the effect of genotype in exon 2 on body weight, carcass weight, weight of breast muscles and weight of wings. Interestingly, some mutations in the myostatin gene affect not only muscling, but also other parameters such bird mortality, which may be indicative of a pleiotropic effect of the gene (Ye et al., 2007).

In summing up, no birds with g.2242C>G mutation were identified among Ross 308 chickens. The population was monomorphic and only represented by birds of CC genotype. g.2373C>T polymorphism was identified. Cockerels with CT (0.43) and CC genotypes (0.36) were most frequent. Allele T with substitution had a frequency of 0.43. g.2373C>T polymorphism in the myostatin gene did not determine the growth parameters and dressing percentage in Ross 308 broiler chickens.

References

Baron E.E., Wenceslau A.A., Alvares L.E., Nones K., Ruy D.C., Schmidt G.S., Zanel-la E.L., Coutinho L.L., Ledur M.C. (2002). High level of polymorphism in the myostatin chicken gene. Proc. 7th World Congr Genet Appl Livest Prod Montpellier, ss. 19–23.

Elkasraw y M.N., Hamrick M.W. (2010). Myostatin (GDF-8) as a key factor linking muscle mass and skeletal form. J. Musculoskelet. Neuronal. Interact. 10, 1: 56–63.

Genxi M.J., Guojun D., Jinyu W., Yue W., Fuxiang L., Zhang L., Xiuhua Z., Kaiz-hou X., Wenhao W. (2014). Polymorphisms in 5' upstream region of the myoststin gene in four chicken breeds and its relationship with growth traits in the Bian chicken. Afr. J. Biotech., 11: 9677–9682.

Han J., Forrest R.H., Hickford J.G. (2013). Genetic variations in the myostatin gene (MSTN) in New Zealand sheep breeds. Mol. Biol. Rep., 40: 6379–6384.

Hill E.W., Gu I. J., Eivers S.S., Fonseca 1 R.G., McGivney B.A., Govindarajan P., Orr N., Katz L.M., MacHugh D. (2010). A sequence polymorphism in MSTN predicts sprinting ability and racing stamina in Thoroughbred horses. PLOS Articles 5, 1: 1–6.

Khaerunnisa I., Pramujo M., Arief I.I., Budiman C., Gunawan A., Sumantri C. (2016). Polymorphism of the T4842G myostatin gene is associated with carcass characteristics in Indonesian chicken. In. J. Poult. Sci., 8: 316–324.

Kumar S. T., Dilbaghi N., Ahlawat S.P.S., Mishra B., Tantia M.S., Vijh R.K. (2007). Genetic relationship among chicken populations of India based on SNP markers of myostatin gene GDF 8. J. Poult. Sci., 6: 684–688.

Kwasek M. (2013). Tendencje w spożyciu mięsa na świecie. Rocz. Ekon. KPSW w Bydgoszczy, 6: 265–284.

McPherron A.C., Lee S.J. (1997). Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA, 94: 12457–12461.

Mott Ivarie R. (2002). Expression of myostatin is not altered in lines of poultry exhibiting myofiber hyper- and hypoplasia. Poultry Sci., 81: 799–804.

Nowak M., Trzis z k a T. (2010). Zachowania konsumentów na rynku mięsa drobiowego. Zywn.--Nauk. Technol. Ja., 68: 114–120.

Paswan C., Bhattacharya T.K., Nagaraj C.S., Chaterjee R.N., Jayashankar M.R. (2014). SNPs in minimal promoter of myostatin (GDF-8) gene and its association with body weight in broiler chicken. J. App. Anim. Res., 42: 304–309.

Ye X., Brown S.R., Nones K., Coutunho L.L., Dekkers J.C.M., Lamont S.J. (2007). Asso-ciations of myostatin gene polymorphisms with performance and mortality traits in broiler chickens. Genet. Selec. Evol. 39, 1: 73–89.

Zhang G., Ding F., Wang J., Dai G., Xie K., Zhang L., Wang W., Zhou S. (2011). Poly-morphism in exons of the myostatin gene and its relationship with body weight traits in the Bian chicken. Biochemical. Genet., 49: 9–19.

Z h a n g G.X., Z h a o X.H., Wa n g J.Y., D i n g F.X., Z h a n g L. (2012). Effect of an exon 1 mutation in the myostatin gene on the growth traits of the Bian chicken. Anim. Genet., 43, 4: 458–459.
Z h i l i a n g G. D a h a i Z., N i n g L., H u i L., X u e m e i D., C h a n g x i n W. (2004). The single nucleotide polymorphisms of the chicken myostatin gene are associated with skeletal muscle and adipose growth. Sci. China Life. Sci., 47: 25–30.
Z h a n g L., T a n g M., Ya n g J., Wa n g O., C a i C., J i a n g S., L i H., J i a n g K., G a o P., M a D., C h e n X., A n X., L i 1 K., C u i W. (2015). Targeted mutations in myostatin by zinc-finger nucleases result in double-muscled phenotype in Meishan pigs. Sci. Rep., 5, 14435: 1–13.

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Summary

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