

## Examination of Growth Hormone (*GH*) Gene Polymorphism and its Association with Body Weight and Selected Body Dimensions in Ducks

Artur MAZUROWSKI, Anna FRIESKE, Dariusz KOKOSZYŃSKI, Sławomir MROCZKOWSKI,  
Zenon BERNACKI, and Anna WILKANOWSKA

Accepted December 15, 2014

MAZUROWSKI A., FRIESKE A., KOKOSZYŃSKI D., MROCZKOWSKI S., BERNACKI Z., WILKANOWSKA A. 2015. Examination of growth hormone (*GH*) gene polymorphism and its association with body weight and selected body dimensions in ducks. *Folia Biologica (Kraków)* **63**: 43-50.

The main objective of the study was to assess the polymorphism in intron 2 of the *GH* gene and its association with some morphological traits (body weight- BW, length of trunk with neck- LTN, length of trunk- LT, chest girth- CG, length of breast bone- LBB, length of shank- LS). Polymorphism in intron 2 of the *GH* gene was evaluated for four duck populations (Pekin ducks AF51, Muscovy ducks from a CK and CRAMMLCFF mother and Mulard ducks). Genetic polymorphism was determined with the PCR-RFLP method using the *BsmFI* restriction enzyme. In the studied duck sample two alleles ( $GH^C$  and  $GH^T$ ) and three genotypes ( $GH/TT$ ,  $GH/CT$ ,  $GH/CC$ ) were found at locus *GH/BsmFI*. In both groups of Muscovies and in Mulards the dominant allele was  $GH^T$ . On the contrary in Pekin ducks AF51, the frequency of both alleles was found to be similar. The most frequent genotype in the examined ducks was  $GH/TT$ . In Pekin ducks AF51 three genotypes were observed, while in Mulard ducks and in male Muscovy ducks from a mother marked as CK, two genotypes ( $GH/TT$  and  $GH/CT$ ) were identified. Muscovy duck females from a CK mother and all males and females of Muscovy duck from a CRAMMLCFF mother were monomorphic with only the  $GH/TT$  genotype detected. The results showed that males of Pekin duck AF51 with the  $GH/TT$  genotype were characterized by higher ( $P<0.01$ ) BW value than those with the  $GH/CC$  and  $GH/CT$  genotype. In females of Pekin ducks AF51, this same trend was observed; individuals with  $GH/TT$  genotype were superior ( $P<0.05$  and  $P<0.01$ ) to birds with two other detected genotypes in respect to BW, CG, LBB and LS. In the case of Mulards, ducks with the  $GH/TT$  genotype were distinguished by higher values of all evaluated traits compared to ducks with  $GH/CT$  and  $GH/CC$  genotypes, however most of the recorded differences were not significant. The only trait markedly impacted ( $P<0.05$ ) by the polymorphism of the *GH* gene intron 2 was the LS value in males.

Key words: Ducks, *GH* gene, genetic polymorphism, body measurements.

Artur MAZUROWSKI, Sławomir MROCZKOWSKI, Department of Genetics and General Animal Breeding, University of Technology and Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland.

E-mail: artur.mazurowski@utp.edu.pl

Anna FRIESKE, Dariusz KOKOSZYŃSKI, Zenon BERNACKI, Department of Poultry Breeding and Evaluation of Animal Products, University of Technology and Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland.

Anna WILKANOWSKA, Department of Agricultural, Environmental and Food Sciences, University of Molise, Via F. de Sanctis snc, 86100 Campobasso, Italy.

Advances in molecular genetics of livestock animals have led to the identification of genes or markers associated with genes that influence growth, carcass and meat quality traits, but also reproductive features (CHANG *et al.* 2012; DVOŘÁKOVÁ *et al.* 2012; BHATTACHARYA & CHATTERJEE 2013, HUANG *et al.* 2013). The molecular basis of these characteristics is being revealed by functional genomics methods, giving opportunities to enhance genetic improvement programs in farm animals through marker-assisted selection (MAS) (GAO *et al.* 2007).

Several genes have been used as candidate genes for marker-assisted selection for improved productive and reproductive performance of animals; one of these genes is the growth hormone gene (*GH*) (SUPAKORN & PRALOMKARN 2013). The expressed product of the *GH* gene is the protein growth hormone (GH) (also called somatotropin), a member of the growth hormone/prolactin family, produced in specific cells (somatotrophs) of the pituitary gland (WALLIS 1988). It has many physiological functions, such as promoting muscle growth (GE *et al.* 2012), bone growth and develop-

ment (OHLSSON *et al.* 1998), regulation of fat content (ZHANG *et al.* 2007) and metabolism (BAUMAN 1999). In addition, GH plays an important role in innate and acquired immune systems. It has been proven to affect the proliferation of lymphoid cells, the activity of phagocytic cells, thymulin excretion and the growth of the thymus (GALA 1991). Studies in animals indicate that GH is involved in the processes of sexual differentiation, pubertal maturation and participates in gonadal steroidogenesis, gametogenesis and ovulation (HULL & HARVEY 2001). In birds, GH has an important function in growth but is also involved in a variety of secondary functions such as egg production, aging and reproduction (KANSAKU *et al.* 2003).

Due to this functional importance, the *GH* gene has been studied in a wide range of species. The genomic structure of this gene has been examined among others in fish (DU *et al.* 1993), rat (BARTA *et al.* 1981), mouse (DAS *et al.* 1996), bovids (WOYCHIK *et al.* 1982), sheep (BYRNE *et al.* 1987), pig (VIZE & WELLS 1987) and human (FIDDES *et al.* 1979). In birds, *GH* cDNA clones have been isolated and sequenced from chicken (LAMB *et al.* 1988), turkey (FOSTER *et al.* 1990) and duck (CHEN *et al.* 1988). The genomic sequence of the avian *GH* gene was first reported in chicken (TANAKA *et al.* 1992).

In all mammals the *GH* gene extends over 2-3 kb and comprises five exons split by four introns (JING *et al.* 2006). The duck *GH* gene is 5.25 kb in size, also consists of five exons and four introns, and is structurally similar to the mammalian and chicken *GH* genes (KANSAKU *et al.* 2008). Furthermore, the *GH* gene is highly polymorphic in a variety of livestock animals. Many polymorphisms have been identified in the *GH* gene of pig (WENJUN *et al.* 2003), bovids (LUCY *et al.* 1993), goat (MALVEIRO *et al.* 2001) and poultry (SHENG-HAI *et al.* 2007; GHELGHACHI *et al.* 2013; ZHANG *et al.* 2014). In ducks, the effect of *GH* gene polymorphism on important economic traits has been noted (WU *et al.* 2012; 2014). Moreover, in a study conducted on ducks by HIYAMA *et al.* (2012) it was suggested that variation in the promoter of *GH* may influence laying performance by changing the expression of GH mRNA levels in the anterior pituitary gland.

The objectives of this study were to estimate the allele and genotype frequencies of *GH/BsmFI* polymorphism of Pekin ducks strain AF51, Muscovy and Mulard ducks. Additionally, we investigated the possible associations of duck growth hormone gene polymorphism with body weight and some body dimensions of ducks in order to identify a potential marker to be used as a complementary parameter in the selection of ducks.

## Material and Methods

### Animals

The experiment was conducted at the Mochelek Experiment Station in the Kuyavian-Pomeranian Province and in the Faculty of Animal Breeding and Biology at the University of Technology and Life Sciences in Bydgoszcz (Poland). The tested material consisted of 47 Pekin ducks strain AF51 (30 males, 17 females), 51 Muscovies from parents marked as MMLCFFCZK'CK (24 males, 27 females), 46 Muscovies from MMLCFFCZK'CRAMMLCFF parents (21 males, 25 females) and 44 Mulards – set STE MULARD (23 males, 21 females). Ducks were housed in pens according to sex and origin, in a confined building on deep litter. Birds had free access to water. Moreover, during the trial the ducks were fed complete commercial diets *ad libitum* according to age: a starter diet (from 1<sup>st</sup> day to 3<sup>rd</sup> week of age) containing 20.0% CP and 11.7 MJ of metabolizable energy (ME) and a grower/finisher diet (from the 4<sup>th</sup> week of age to the end of the experiment) containing 18.5% CP and 12.2 MJ of ME).

Drakes and ducks were weighed and measured individually at the day of slaughter. The males and females of Pekin ducks AF51 were killed at the 7th week of life, the Muscovy females at the 10th week of life and the Muscovy males and Mulards (males and females) at the 12th week of life. Except for body weight (BW), the following body measurements were taken before slaughter: length of trunk with neck (LTN) – between the first cervical vertebra and base of tail, length of trunk (LT) – between shoulder joint and base of tail, chest girth (CG) – behind the wings, through the anterior border of the breast-bone crest and the central thoracic vertebra, length of breast bone (LBB) – from the anterior to the posterior edge, length of shank (LS) – between the hock joint and bottom posterior area of first toe at its base. Ducks were tape-measured with an accuracy of 0.1 cm.

The study was carried out according to the guidelines of the Ethical Committee in Bydgoszcz (No. 27/2012).

### Detection of polymorphism in intron 2 of the GH gene

Genetic analyses were carried out on genomic DNA isolated from peripheral blood drawn from a wing vein. DNA was isolated from aliquots of 15  $\mu$ l of blood samples using Gene Jet Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, USA). The amount and quality of DNA was measured using a NanoDrop® 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA

of each duck was stored until subjected to allelic discrimination assays at -20°C.

*GH* gene genotypes in intron 2 were determined using the PCR-RFLP method according to WU *et al.* (2012). The PCR product (consisting of 765 base pairs) was digested with 10U of restriction enzyme *Bsm* FI for 4 h in 65°C. The digested fragments were separated on 3% agarose gels with the presence of Midori Green Advanced (Nippon Genetics, Japan). Genotypes were identified against molecular marker O' Gene Ruler Low Range DNA Ladder (Thermo Fisher Scientific, Waltham, USA).

Statistical analysis

Genotype and allele frequencies were calculated in each group of ducks. The data used to compare the effect of polymorphism of *GH* gene on body weight and some biometrical traits of ducks were tested with the use of a model including the effect of each genotype at locus *GH/BsmFI* assessed in Pekin ducks strain AF51 at the 7th week of life, and in Mulard ducks at the 12th week of life. The genetic effects of *GH* gene polymorphism on body weight and some biometrical traits were analyzed using the following model:

$$Y_{ijkl} = \mu + G_i + D_j + S_k + g_{ijkl}$$

Where:

$Y_{ijkl}$  is the observed value of dependent variable,

$\mu$  is the overall mean,

$G_i$  is the fixed effect due to genotype *GH/BsmFI* ( $i = GH/CC, GH/CT, GH/TT$ ),

$D_j$  is the fixed effect due to duck origin ( $j =$  Pekin duck AF51 and Mulard ducks),

$S_k$  is the fixed effect due to sex ( $k =$  males and females),

$g_{ijkl}$  is the random residual error.

Hardy-Weinberg equilibrium was assessed with the use of a Chi square test and the statistical significance of differences among means was calculated in accordance with the SAS Enterprise guide 9.3 software.

Results

Allele frequencies for *GH* gene intron 2 in four duck populations are listed in Table 1. The dominant allele of intron 2 of the duck *GH* gene was  $GH^T$  for three populations: Muscovy ducks (from a CK and CRAMMLCFF mother) and Mulards. No marked differences were observed between frequencies of alleles  $GH^C$  and  $GH^T$  in the Pekin duck strain AF51. The allelic distribution of *GH/BsmFI* polymorphic sites in Muscovy duck populations followed a similar pattern. The results indicate that the  $GH^T$  allele is the preponderant allele in Muscovy duck from a mother marked as CK. Only one allele was found ( $GH^T$ ) in the group of Muscovies from a mother marked as CRAMMLCFF.

As a result of digestion of 765 bp target region of the duck *GH* gene intron 2 by *BsmFI* enzyme, the samples with 765 bp fragment (uncut) were accepted as *GH/TT* genotype, those with 765, 593 and 172 bp fragments as *GH/CT* and those with 593 and 172 bp were evaluated as *GH/CC* genotypes (Fig. 1). The distribution of genotypes of the *GH* gene in the four studied duck populations is presented in Table 2.

The most frequent genotype in the examined duck groups was *GH/TT*. The highest degree of genetic polymorphism for the duck *GH* gene intron 2 was found in Pekin ducks strain AF51. Genotype frequencies of the *GH/BsmFI* locus in this duck population were in Hardy-Weinberg equilibrium ( $P = 0.985$ ).

Table 1

Allele frequencies at intron 2 locus of the *GH* gene depending on origin and sex of ducks

Origin of ducks	Sex	n	Alleles	
			$GH^C$	$GH^T$
Pekin (AF51)	♂	30	0.58	0.42
	♀	17	0.44	0.56
	♂♀	47	0.53	0.47
Muscovy (MMLCFFCZK'CK)	♂	25	0.02	0.98
	♀	27	0.00	1.00
	♂♀	52	0.01	0.99
Muscovy (MMLCFFCZK'CRAMMLCFF)	♂	21	0.00	1.00
	♀	25	0.00	1.00
	♂♀	46	0.00	1.00
Mulard (STE MULARD)	♂	23	0.20	0.80
	♀	21	0.17	0.83
	♂♀	44	0.18	0.82

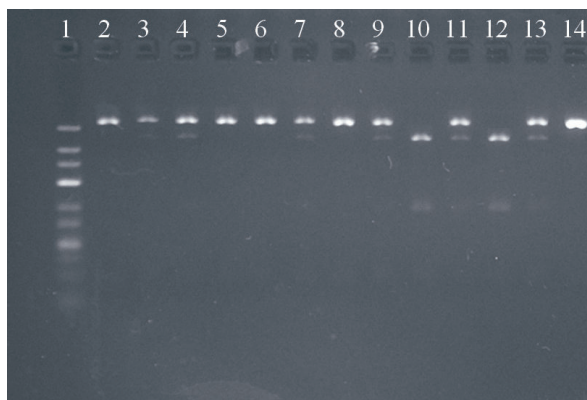


Fig. 1. *GH/BsmFI* genotype identification (line 1 – molecular marker O<sup>+</sup> Gene Ruler Low Range DNA Ladder – 700, 500, 400, 300, 200, 150, 100, 75, 50, 50, 25 bp; lines 2, 3, 5, 6, 8, 14 genotypes *GH/TT* – 765 bp; lines 4, 7, 8, 13 genotypes *GH/CT* – 765, 593 and 172 bp; lines 10, 12 genotypes *GH/CC* – 593 and 172 bp).

Compared to Pekin ducks strain AF51, a lower level of polymorphism for intron 2 of the *GH* gene in the group of Mulard ducks was observed. In this population, two genotypes *GH/TT* and *GH/CT* were identified, with frequencies 0.64 and 0.36, respectively. Moreover, the distribution of genotypes in Mulard ducks was at genetic equilibrium ( $P=0.140$ ).

In terms of genotype frequencies of intron 2 of the *GH* gene, the lowest level of diversity was detected in Muscovy ducks, both CK and CRAMMLCFF. In the group of Muscovy drakes from a CK mother, two genotypes were observed: *GH/TT* (0.96) and *GH/CT* (0.04). In the group of females of Muscovies CK only one genotype was detected (*GH/TT*) and the Chi square test showed that in *GH/BsmFI* locus the distribution of genotypes was in Hardy-Weinberg equilibrium ( $P=0.944$ ). Just as in the case of female Muscovies CK, in the whole group of Muscovy ducks from a CRAMMLCFF mother, monomorphism of the *GH* gene with only the *GH/TT* genotype was found. However, in contrast to Muscovies from a mother marked as CK, genotype frequencies of the *GH* gene deviated from genetic equilibrium ( $P<0.001$ ).

To study the association of the examined *GH* gene (intron 2) polymorphism with morphometric traits we analyzed the relationship between BW and some body measurements with identified genotypes in Pekin ducks AF51 (*GH/CC*, *GH/CT*, *GH/TT*) and Mulards (*GH/CT*, *PRL/TT*). Due to the lack of variability in Muscovies CRAMMLCFF and very low genetic variation in Muscovies CK, we did not evaluate the association of *GH* gene

Table 2

Genotype frequencies at intron 2 locus of the *GH* gene depending on origin and sex of ducks

Origin of ducks	Sex	Number of genotypes (Frequencies of genotypes)						HWE $P_{-value}$
		Observed			Expected			
		<i>GH/TT</i>	<i>GH/CT</i>	<i>GH/CC</i>	<i>GH/TT</i>	<i>GH/CT</i>	<i>GH/CC</i>	
Pekin (AF51)	♂	4.00 (0.13)	17.00 (0.57)	9.00 (0.30)	5.21 (0.17)	14.58 (0.49)	10.21 (0.34)	0.662
	♀	6.00 (0.35)	7.00 (0.41)	4.00 (0.24)	5.31 (0.31)	8.38 (0.49)	3.31 (0.20)	0.794
	♂♀	10.00 (0.21)	24.00 (0.51)	13.00 (0.28)	10.30 (0.22)	23.40 (0.50)	13.30 (0.28)	0.985
Muscovy (MMLCFF CZK' CK)	♂	24.00 (0.96)	1.00 (0.04)	0.00 (0.00)	24.01 (0.96)	0.98 (0.04)	0.01 (0.00)	0.918
	♀	27.00 (1.00)	0.00 (0.00)	0.00 (0.00)	27.00 (1.00)	0.00 (0.00)	0.00 (0.00)	-
	♂♀	51.00 (0.98)	1.00 (0.02)	0.00 (0.00)	51.00 (0.98)	0.99 (0.02)	0.01 (0.00)	0.944
Muscovy (MMLCFF CZK'CRA MMLCFF)	♂	21.00 (1.00)	0.00 (0.00)	0.00 (0.00)	21.00 (1.00)	0.00 (0.00)	0.00 (0.00)	-
	♀	25.00 (1.00)	0.00 (0.00)	0.00 (0.00)	25.00 (1.00)	0.00 (0.00)	0.00 (0.00)	-
	♂♀	46.00 (1.00)	0.00 (0.00)	0.00 (0.00)	46.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-
Mulard (STE MULARD)	♂	14.00 (0.61)	9.00 (0.39)	0.00 (0.00)	14.88 (0.65)	7.24 (0.31)	0.88 (0.04)	0.506
	♀	14.00 (0.67)	7.00 (0.33)	0.00 (0.00)	14.58 (0.69)	5.83 (0.28)	0.58 (0.03)	0.657
	♂♀	28.00 (0.64)	16.00 (0.36)	0.00 (0.00)	29.45 (0.67)	13.09 (0.30)	1.45 (0.03)	0.140



polymorphism in intron 2 with the selected traits in these groups. The results of the statistical analysis of associations between the *GH/BsmFI* polymorphism and body measurements in Pekin duck AF51 are summarized in Table 3. Males of Pekin ducks AF51 with *GH/CC* and *GH/CT* genotype weighed less ( $P<0.01$ ) than males with the *GH/TT* genotype at locus *GH/BsmFI*. Furthermore, the analysis of BW of females from different genetic groups showed that ducks with *GH/TT* and *GH/CT* genotypes were significantly ( $P<0.01$ ) heavier than those with the *GH/CC* genotype. There were no significant ( $P>0.05$ ) differences between both Pekin duck AF51 populations (males and females) with detected genotypes for LTN and LT. Moreover, the CG value in males of the Pekin ducks

AF51 with all identified genotypes was similar ( $P>0.05$ ). Meanwhile, in the case of females, it was observed that individuals with genotypes *GH/CT* and *GH/TT* in comparison with ducks with genotype *GH/CC* were distinguished by a higher ( $P<0.05$  and  $P<0.01$ ) value of CG. The study revealed no significant ( $P>0.05$ ) differences for LBB and LS values between males of Pekin ducks with all detected genotypes. However, the comparison of females with the *GH/CC* genotype and those with the *GH/TT* genotype demonstrated that the latter had markedly higher ( $P<0.05$ ) values of LBB (+9.83%) and LS (+6.79).

Associations of *GH* gene polymorphism in intron 2 with some body measurements of Mulard ducks are presented in Table 4. No differences

Table 3

Association of GH gene polymorphism in 2nd intron with body weight and some body measurements of Pekin ducks strain AF51

Genotype	Sex	Traits					
		BW (g)	LTN (cm)	LT (cm)	CG (cm)	LBB (cm)	LS (cm)
<i>GH/CC</i>	♂	2422.22±179.70 <sup>A</sup>	41.50±0.83	26.94±1.49	36.28±0.66	15.83±0.50	6.28±0.13
	♀	2025.00±36.43 <sup>A</sup>	38.12±0.58	25.75±0.53	32.25±0.56 <sup>Aa</sup>	14.50±0.43 <sup>a</sup>	5.87±0.12 <sup>a</sup>
	♂♀	2300.00±76.79 <sup>Aa</sup>	40.46±0.64	26.58±0.57	35.04±0.68	15.42±0.37	6.15±0.10
<i>GH/CT</i>	♂	2558.33±35.71 <sup>A</sup>	42.25±0.63	27.00±0.63	36.83±0.53	16.48±0.39	6.33±0.10
	♀	2485.71±20.07 <sup>B</sup>	39.28±0.49	26.64±0.36	35.64±0.71 <sup>b</sup>	15.50±0.24	6.28±0.11
	♂♀	2519.23±23.02 <sup>b</sup>	40.65±0.58	28.81±0.37	36.19±0.45	15.95±0.27	6.31±0.07
<i>GH/TT</i>	♂	2825.00±77.19 <sup>B</sup>	40.75±0.55	27.37±0.17	38.25±0.78	16.12±0.24	6.37±0.11
	♀	2625.00±92.42 <sup>B</sup>	40.08±0.59	27.25±0.83	36.08±1.03 <sup>B</sup>	16.08±0.43 <sup>b</sup>	6.33±0.12 <sup>b</sup>
	♂♀	2705.00±64.74 <sup>B</sup>	40.35±0.40	27.30±0.32	36.95±0.68	16.10±0.21	6.35±0.08
P-value	♂	<0.01	ns	ns	ns	ns	ns
	♀	<0.01	ns	ns	<0.01	<0.05	<0.05
	♂♀	<0.05	ns	ns	ns	ns	ns

BW – Body weight, LTN – Length of trunk with neck, LT – Length of trunk, CG – Chest girth, LBB – Length of breast bone, LS – Length of shank,

<sup>ABC</sup> Values within a row followed by different letters differ significantly ( $P<0.01$ )

abc Values within a row followed by different letters differ significantly ( $P<0.05$ ), ns –  $P>0.05$ .

Table 4

Association of GH gene polymorphism in 2nd intron with body weight and some body dimensions of Mulard ducks

Genotype	Sex	Traits					
		BW (g)	LTN (cm)	LT (cm)	CG (cm)	LBB (cm)	LS (cm)
<i>GH/CT</i>	♂	3272.22±128.59	41.78±0.54	29.94±0.58	39.83±0.77	18.44±0.22	6.94±0.05
	♀	2842.86±88.93	41.50±0.47	28.71±0.53	38.07±0.89	17.64±0.26	6.28±0.10
	♂♀	3884.37±96.79	41.66±0.36	29.41±0.42	39.06±0.61	18.09±0.19	6.66±0.09
<i>GH/TT</i>	♂	3318.18±108.98	42.36±0.47	30.04±0.54	41.14±0.42	18.59±0.21	7.07±0.08
	♀	2857.69±97.54	42.07±0.47	28.92±0.55	38.38±0.60	18.11±0.25	6.61±0.08
	♂♀	3068.75±85.69	42.21±0.33	29.44±0.40	39.64±0.47	18.33±0.17	6.81±0.07
P-value	♂	ns	ns	ns	ns	ns	<0.05
	♀	ns	ns	ns	ns	ns	ns
	♂♀	ns	ns	ns	ns	ns	ns

BW – Body weight, LTN – Length of trunk with neck, LT – Length of trunk, CG – Chest girth, LBB – Length of breast bone, LS – Length of shank, ns –  $P>0.05$ .

( $P > 0.05$ ) were observed in BW, LTN, LT, CG and LBB values between both Mulard duck populations (males and females) with identified genotypes. No significant differences were found for the LS value between females with the *GH/CT* and *GH/TT* genotypes. However, considering the effect of *GH* gene polymorphism on LS of drakes, it was observed that this value was higher for males with *GH/TT* genotype ( $P < 0.05$ ) than for the drakes with genotype *GH/CT* (+1.84%).

## Discussion

In the present experiment the polymorphism of intron 2 of the duck *GH* gene was examined. Previous studies had shown that the polymorphisms of the avian *GH* gene can be identified not only at intron 2. Polymorphisms in exon regions of this gene were detected, among others, in ducks (CHANG *et al.* 2012) and geese (ZHANG *et al.* 2014). Meanwhile, polymorphisms in intronic regions of the avian *GH* gene were found in chickens at intron 1 (GHELGHACHI *et al.* 2013), at intron 2 in ducks (WU *et al.* 2012), at intron 3 in geese (ZHAO *et al.* 2008) and chicken (ZHANG *et al.* 2007), but also at intron 4 in chickens (NIE *et al.* 2002).

There is little information in the literature concerning identification of *BsmFI* polymorphisms in the 2<sup>nd</sup> intron of duck *GH* gene. However, it is possible to compare the frequency of *GH* gene alleles and genotypes presented herein with results described by WU *et al.* (2012). The allele frequencies reported in the above-mentioned study, which was carried out in three duck populations, differs from the one recorded in our experiment. Moreover, the present results were not in accordance with the findings in WU *et al.* (2012) which reported that the usage of *BsmFI* restriction enzyme in all duck populations enabled the detection of three genotypes. The observed differences may result from the origin of experimental animals. In the current study, the monomorphism of *GH* gene in second intron observed in the whole group of Muscovy ducks CRAMMLCFF may have resulted from the homozygosity of parents.

Productive performance of poultry is affected by quantitative traits that can be influenced by many environmental factors and genes, such as the growth hormone gene. *GH* gene polymorphisms have been studied in various poultry species, such as chicken (ZHANG *et al.* 2007), quail (JOHARI *et al.* 2013), goose (ZHAO *et al.* 2011) and ducks (WU *et al.* 2014). In these poultry species a high degree of polymorphism of the DNA sequence of the *GH* gene was detected. CHANG *et al.* (2012) found 19 SNPs in a region of 2087 base pairs (bp) in the duck *GH* gene. This study indicated that each SNP

was associated with at least one duck fertility-related trait. However, available specialist literature shows that some SNPs in the duck *GH* gene affected also growth and carcass traits. The effect of avian growth hormone gene on the above-mentioned characteristics was recorded by WU *et al.* (2012), who as the first discovered *BsmFI* polymorphism in duck *GH* gene in the second intron. This Chinese research group tested three different breeds of duck (Cherry Valley, Muscovy, Jingjiang) slaughtered after 56 days of life. Considering body weight of ducks at the day of slaughter, the results of the present study partially confirm the observations of WU *et al.* (2012). They noted that in one of the evaluated duck breeds (Jingjiang), individuals with genotype *GH/TT* were heavier than those with *GH/CT* and *GH/CC* genotypes. In the present study we recorded this same trend in both Pekin AF51 and Mulard ducks. However, our findings regarding two other breeds are not in agreement with those described by WU *et al.* (2012). In the groups of Cherry Valley and Muscovies, the heavier birds were the ones with the genotype *GH/CT* (WU *et al.* 2012).

Because of a lack of any comparable results concerning the effect of the *GH* gene on body measurements in duck, the verification of our findings with those in previous literature is hampered. However, the results of our study proved that in comparison to ducks of other genetic groups, individuals with the *GH/TT* genotype displayed higher values of most of the assessed features, which indicates that the *GH* gene may be a candidate for a marker of biometrical traits in ducks.

In conclusion, the highest degree of polymorphism in the second intron of the *GH* gene was observed in Pekin ducks strain AF51, while the population of Muscovy ducks from a CRAMMLCFF mother was monomorphic. The results of this study with regard to *GH/BsmFI* genotype showed a significant influence on some biometrical traits such as values of BW, CG, LBB, LS in Pekin ducks AF51 and LS in Mulard ducks. *GH*

## References

- BARTA A., RICHARDS R.I., BAXTER J.D., SHINE J. 1981. Primary structure and evolution of rat growth hormone gene. *Proc. Natl. Acad. Sci.* **78**: 4867-4871.
- BAUMAN D.E. 1999. Somatotropin mechanism in lactating cows: from basic science to commercial application. *Domest. Anim. Endocrinol.* **17**: 101-116.
- BHATTACHARYA T.K., CHATTERJEE R. N. 2013. Polymorphism of the myostatin gene and its association with growth traits in chicken. *Poult. Sci.* **92**: 910-915.
- BYRNE C.R., WILSON B.W., WARD K.A. 1987. The isolation and characterization of the ovine growth hormone gene. *Aust. J. Biol. Sci.* **40**: 459-468.

- CHANG M.-T., CHENG Y.-S., HUANG M.-C. 2012. The SNP genotypes of growth hormone gene associated with reproductive traits in Tsaiya ducks. *Reprod. Dom. Anim.* **47**: 568-573.
- CHEN H.T., PAN F.M., CHANG W.C. 1988. Purification of duck growth hormone and cloning of the complementary DNA. *Biochim. Biophys. Acta.* **949**: 247-251.
- DAS P., MEYER L., SEYFERT H.-M., BROCKMANN G., SCHWERIN M. 1996. Structure of the growth hormone-encoding gene and its promoter in mice. *Gene* **169**: 209-213.
- DU S.J., DEVLIN R.H., HEW C.L. 1993. Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, *GH-I* and *GH-II*, and a male-specific pseudogene, *GH-psi*. *DNA Cell Biol.* **12**: 739-751.
- DVOŘÁKOVÁ V., BARTENSCHLAGER H., STRATIL A., HORÁK P., STUPKA R., ČITEK J., ŠPŘYSL M., HRDLICOVÁ A., GELDERMANN H. 2012. Association between polymorphism in the *FTO* gene and growth and carcass traits in pig crosses. *Genet. Sel. Evol.* **44**: 13.
- FIDDES J.C., SEEBURG P.H., DENOTO F.M., HALLEWELL R.A., BAXTER J.D., GOODMAN H.M. 1979. Structure of genes for human growth hormone and chorionic somatomotropin. *Proc. Natl. Acad. Sci.* **76**: 4294-4298.
- FOSTER D.N., KIM S.U., ENYEART J.J., FOSTER L. K. 1990. Nucleotide sequence of the complementary DNA for turkey growth hormone. *Biochem. Biophys. Res. Comm.* **173**: 967-975.
- GALA R.R. 1991. Prolactin and growth hormone in the regulation of the immune system. *Exp. Biol. Med.* **198**: 513-527.
- GAO Y., ZHANG R., HU X., LI N. 2007. Application of genomic technology to the improvement of meat quality of farm animals. *Meat Sci.* **77**: 36-45.
- GAO Y., LIU D., MA W., LI A., LAN X., ZHANG C., LEI C., CHEN H. 2013. Two novel SNPs in the coding region of bovine *VDR* gene and their associations with growth traits. *J. Genet.* **92**, e53-e59. Online: <http://www.ias.ac.in/jgenet/OnlineResources/92/e53.pdf>. 25 June 2013.
- GE X., YU J., JIANG H. 2012. Growth hormone stimulates protein synthesis in bovine skeletal muscles cells without altering insulin-like growth factor-I mRNA expression. *J. anim. Sci.* **90**: 1126-1133.
- GHELGHACHI A.A., REZA SEYEDABADI H., LAK A. 2013. Association of growth hormone gene polymorphism with growth and fatness traits in Arian broilers. *Int. J. Biosci.* **3**: 216-220.
- HIYAMA G., OKABAYASHI H., KANSAKU N., TANAKA K. 2012. Genetic variation in the growth hormone promoter region of *Anas platyrhynchos*, a duck native to Myanmar. *J. Pout. Sci.* **49**: 245-248.
- HUANG Y.Z., HE H., ZHAN Z.Y., SUN Y.J., LI M.X., LAN X.Y., LEI C.Z., ZHANG C.L., CHEN H. 2013. Relationship of polymorphisms within *ZBED6* gene and growth traits in beef cattle. *Gene* **526**: 107-111.
- HULL K.L., HARVEY S. 2001. Growth hormone: roles in female reproduction. *J. Endocr.* **168**: 1-23.
- JOHARI S., SETIATI N., SIDADOLOG J.H.P., HARTATIK T., YUWANTA T. 2013. The gene effect of growth hormone on body weight and egg production in divergent selection for five generation of Japanese quail (*Coturnix coturnix japonica*). *Inter. J. Poult. Sci.* **12**: 489-494.
- JING L.I., RAN X.-Q., WANG J.-F. 2006. Identification and function of the growth hormone gene in Rongjiang pig of China. *Acta Phys. Sin.* **58**: 217-224.
- KANSAKU N., NAKADA A., OKABAYASHI H., GUÉMÉNÉ D., KÜHNLEIN U., ZADWORNÝ D. SHIMADA K. 2003. DNA polymorphism in the chicken growth hormone gene: Association with egg production. *Anim. Sci. J.* **74**: 243-244.
- KANSAKU N., SOMA A., FURUKAWA S., HIYAMA G., OKABAYASHI H., GUÉMÉNÉ D., KÜHNLEIN U., ZADWORNÝ D. 2008. Sequence of the domestic duck (*Anas platyrhynchos*) growth hormone-encoding gene and genetic variation in the promoter region. *Anim. Sci. J.* **79**: 163-170.
- LAMB I.C., GALEHOUSE D.M., FOSTER D.N. 1988. Chicken growth hormone cDNA sequence. *Nucleic Acids Res.* **16**: 9339.
- LUCY M.C., HAUSER S.D., EPPARD P.J., KRIVI G.G., CLARK J.H., BAUMAN D.E., COLLIER R.J. 1993. Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. *Domest. Anim. Endocrinol.* **10**: 325-333.
- MALVEIRO E., PEREIRA M., MARQUES P.X., SANTOS I.C., BELO C., RENAUVILLE R., CRAVADOR A. 2001. Polymorphisms at the five exons of the growth hormone gene in the Algarvia goat: possible association with milk traits. *Small Rumin. Res.* **41**: 163-170.
- NIE Q., IP S.C.Y., ZHANG X., LEUNG F.C., YANG G. 2002. New variations in intron 4 of growth hormone gene in Chinese Native Chickens. *J. Hered.* **93**: 277-279.
- OHLSSON C., BENGTSSON B.A., ISAKSSON O.G., ADREASSEN T.T., SLOOTWEG M.C. 1998. Growth hormone and bone. *Endocr. Rev.* **19**: 55-79.
- SHENG-WAI X., WEN-BIN B., JUN H., JUN-HUA C., JING-TING S., GUA-HONG C. 2007. Polymorphic analysis of intron 2 and 3 of growth hormone gene in duck. *Hereditas* **29**: 438-442.
- SUPAKORN C., PRALOMKARN W. 2013. Genetic polymorphisms of growth hormone (*GH*), insulin-like growth factor 1 (*IGF-1*) and diacylglycerol acyltransferase 2 (*DGAT-2*) genes and their effect on birth weight and weaning weight in goats. *Philipp. Agric. Scientist.* **96**: 18-25.
- TANAKA M., HOSOKAWA Y., WATAHIKI M., NAKASHIMA K. 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. *Gene* **112**: 235-239.
- VIZE P.D., WELLS J.R. 1987. Isolation and characterization of the porcine growth hormone gene. *Gene* **55**: 339-344.
- WALLIS M. 1988. Mechanism of action of growth hormone. (In: *Hormones and Their Actions. Part 2: Specific Action of Protein Hormones.* COOKE B.A., KING R.J.B., VAN DER MOLEN H.J. eds, Elsevier, Biomedical Division): 265-272.
- WENJUN W., LUSHENG H., JUN G., NENGSHUI D., KEFEI C., JUN R., MING L. 2003. Polymorphism of Growth Hormone Gene in 12 Pig Breeds and Its Relationship with Pig Growth and Carcass Traits. *Asian-Aust. J. Anim. Sci.* **16**: 161-164.
- WOYCHIK R.P., CAMPER S.A., LYONS R.H., HOROWITZ S., GOODWIN E.C., ROTTMAN F.M. 1982. Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Res.* **10**: 7197-7210.
- WU Y., PAN A.L., PI J.S., PU Y.J., DU J.P., LIANG Z.H. SHEN J. 2012. One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. *Mol. Biol. Rep.* **39**: 8027-8033.
- WU X., YAN M.J., LIAN S.Y., LIU X.T., LI A. 2014. *GH* gene polymorphism and expression associated with egg laying in Muscovy ducks (*Cairina moschata*). *Hereditas* **151**: 14-19.

- ZHANG X. L., JIANG X., LIU Y. P., DU H. R., ZHU Q. 2007. Identification of A<sub>1</sub> polymorphisms in the third intron of *GH* gene and their associations with abdominal fat in chickens. *Poult. Sci.* **86**: 1079–1083.
- ZHANG Y., ZHU Z., XU Q., CHEN G. 2014. Association of polymorphisms of exon 2 of the growth hormone gene with production performance in Huoyan goose. *Int. J. Mol. Sci.*, **15**: 670-683.
- ZHAO W., CHEN Q., CHENG J., WU X., CHEN G. 2008. Polymorphism of intron 3 of growth hormone gene and its relationship with body weight and carcass traits in Zi geese. *Chin. J. Anim. Vet. Sci.* **4**: 443-448.
- ZHAO W., ZHAO R., QIAO N., XU Q., HUANG Z., LI X., ZHANG Y., CHEN G. 2011. Association of *GH* polymorphisms with growth traits in goose. *J. Anim. Vet. Adv.* **10**: 692-697.