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Polymorphisms in Intron 2 of Growth Hormone Gene and Their Associations with Economic Traits in Muscovy, Pekin, and Mulard Ducks

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Abstract **Original Research Article**

This study aimed to determine the effects of genetic variability or polymorphisms in intron 2 of the *GH* gene in three duck populations—Muscovy, Pekin, and Mulard. We employed PCR–RFLP to detect the various genotypes and studied their associations with important economic traits, such as body, dressing, breast, and thigh weights. Two alleles, *GHT* and *GHC*, and three genotypes, *GH/TT*, *GH/CT*, and *GH/CC*, were identified in ducks at the locus, *GH/BsmFI*. In all duck populations, the frequency of allele T was higher than that of allele C and the most frequent genotype was *GH/TT*. All three genotypes were detected in Pekin ducks; whereas, only *GH/TT* and *GH/CT* genotypes were observed in Muscovy and Mulard ducks. Ducks with *GH/TT* genotype was superior ($P < 0.001$) to birds with other genotypes with respect to the body, dressing, and breast muscle weights. Muscovy ducks with *GH/TT* genotype had greater thigh weight ($P < 0.001$) than those with *GH/CT* genotype. However, no significant difference was detected for thigh weight among the three genotypes in Pekin and Mulard ducks. Based on our study, we concluded that the *GH* gene is an excellent genetic marker for enhancing the genetic potential of ducks for economically important traits.

Keywords: Ducks; economic traits; *GH* gene, polymorphism; PCR–RFLP.

INTRODUCTION

Growth rate and meat quality are two economically significant traits in ducks reared for meat. These are the primary concerns in breeding programs for determining their economical values [1, 2]. This has subsequently spurred poultry scientists and producers to incorporate significant traits during selection in breeding programs to increase growth rates. The candidate gene approach stands out among the most imperative techniques to recognize DNA markers associated with economically important traits in farm animals [3]. Once a DNA polymorphism is associated with an important trait, the DNA marker could be used in the molecular MAS [4]. Several genes have been used as candidates for detecting polymorphisms related to productive and reproductive performances in animals; one of these is the growth hormone (*GH*) gene [5].

Growth hormone (GH) is a polypeptide hormone, created and secreted by the somatotroph cells of the anterior lobe of the pituitary gland, in a circadian and pulsatile manner [6]. It is necessary for growth and development [7], bone composition [8], lactation [9], reproduction [10] as well as metabolism of carbohydrates, lipids and proteins [11, 12]. Besides, *GH* has a crucial role in innate and acquired immune responses. It affects the activity of phagocytic cells, the proliferation of lymphoid cells, thymus growth, and thymulin excretion [13]. Recent studies indicated that *GH* is involved in sexual differentiation, pubertal maturation, gonadal steroidogenesis, gametogenesis, and ovulation [14,15]. In birds, *GH* is not only essential for growth but is also embedded in a variety of secondary functions such as egg production, aging, and reproduction [16].

So far, the genomic structure of the *GH* gene, which directly controls the synthesis of *GH*, has been studied in many species. The *GH* gene is conserved in

all the mammals as well as chicken, sharing a similar gene structure of five exons and four introns [17]. Furthermore, many polymorphisms have been identified in the GH genes of pigs [18, 19], bovines [20, 21], goats [22] and poultry [23-25]. Accordingly, the objectives of our study were to detect polymorphisms in the second intron of *cGH* gene using the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method and to analyze the associations of these polymorphisms with some economic traits in three duck populations—Muscovy, Pekin, and Mulard ducks.

MATERIALS AND METHODS

Duck populations and phenotypic data

One-day-old, male ducklings of Muscovy (n = 40), Pekin (n = 40) and Mulard (n = 40) breeds were obtained from a local, commercial hatchery and raised on floor pens with wood shavings as bedding, with a bird density of 5 ducks m^{-2}. Each duck was fitted with a wing band. During the trial, the environmental temperature was maintained at 23 ±2.5 °C, and the light was provided continuously. From 1st to 4th weeks of age, the ducklings were fed with pelleted starter diets consisting of 22% crude protein (CP) and 12.12 MJ of metabolizable energy. From 5th to 12th weeks of age, they were fed with pelleted grower/finisher diets with 20% CP and 12.56 MJ of metabolizable energy. Ducks had ad libitum access to food and water. The experimental protocol was in accordance with the regulations of the Local Experimental Animals Care Committee (ANWD 206) and approved by the institutional ethics committee. No mortality or morbidity was recorded during the study.

At the age of 12 weeks, body weights (BW) of live birds were measured, after 12 h with no access to feed. Their blood samples were harvested from the brachial vein into sterile vacutainer tubes containing the anticoagulant, EDTA, and then stored at −20 °C until genomic DNA extraction. The birds were then euthanized by cervical dislocation, manually feathered, processed by removing the head, neck, shanks, and feet, and eviscerated. The dressed carcass, breast muscle, and thigh muscle were weighed and their percentages relative to live body weights were calculated.

Genomic DNA extraction

Genomic DNA was extracted from blood samples, using GeneJET whole blood genomic DNA purification mini kit (Thermo Fisher Scientific, Waltham, USA), following the manufacturers protocol. The quality and quantity of the resulting DNA from each sample was measured using agarose gel (0.8%) electrophoresis and NanoDrop®2000 spectrophotometer (Thermo Scientific, Waltham, USA), respectively. Genomic DNA from each duck was stored at –20 °C prior to the allelic discrimination assays.

PCR amplification

A fragment of 673 bp covering intron 2 in *GH* gene was amplified with the following sequences: F:5′–GGAGGGCTAAGATCGTGCAT–3′ and R:5′–GCTTGTCCAGGGGAGACAAC–3′ [2]. The qRT–PCR was performed using 25 µL reactions, containing 12.5 µL DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, USA), 2 µL cDNA template, 1 µL of each primer (10 pmol/µL), and 8.5 µL deionized water. Following cycling conditions were applied: 5 min at 94 °C, followed by 40 cycles of 20 s at 95 °C, 20 s at 60 °C, and 30 s at 72 °C, with a final extension of 5 min at 72 °C. The amplified fragments were separated on 1.5% agarose gel electrophoresis and visualized in a gel documentation system under UV transilluminator (Bio-Doc Analyse, Biometra, Germany).

PCR–RFLP analysis

About 5 µL of the PCR product was digested with 10 units of BsmFI restriction enzyme (Fermentas, Shenzhen, China) for 4 h at 65ºC. The digested fragment was identified by electrophoresis using 1.5% agarose gel, stained with ethidium bromide, and visualized in a gel documentation system under UV light. Genotypes were identified against the molecular marker, O'GeneRuler Low Range DNA ladder (Thermo Fisher Scientific, Waltham, USA).

STATISTICAL ANALYSES

All analyses were performed using SAS statistical system package v9.1 [26]. The allelic and genotypic frequencies were defined according to Falconer and Mackay [27], and Hardy–Weinberg equilibrium was explored by the chi-square test. Marker¬trait association analysis was conducted using t-test procedures in Muscovy and Mulard ducks and GLM procedures in Pekin ducks. The model used for data analysis was as follows: $Yij = \mu + Gi + Dj + eij$

where, Yij = observation of the target trait, µ = overall mean, Gi = fixed effect of ith genotype (*GH/TT*, *GH/CT*, and *GH/CC*), Dj = fixed effect of jth duck population (Muscovy, Pekin, and Mulard), and eij = random residual error.

The values were presented as least square means ±SE. Bonferroni test was used to determine significant differences between mean values in Muscovy ducks. The effects of allelic substitution and dominance were calculated. The genotypes, *GH/TT*, *GH/CT*, and *GH/CC*, were represented as 0, 1, and 2, respectively, for the allelic substitution effect, while they were indicated as 0, 1, and 0, respectively, for the dominance effect.

RESULTS

PCR–RFLP analysis and allelic and genotypic frequencies of GH gene intron 2

The amplified genomic DNA of the *GH* gene produced a 673 bp fragment, covering intron 2 (Fig. 1). Three different genotypes were detected as a result of the digestion of this fragment with the *BsmFI* restriction enzyme. The genotypes are designated as TT (uncut 673 bp), CT (673, 456, and 217 bp), and CC (456 and 217 bp) (Fig. 2).

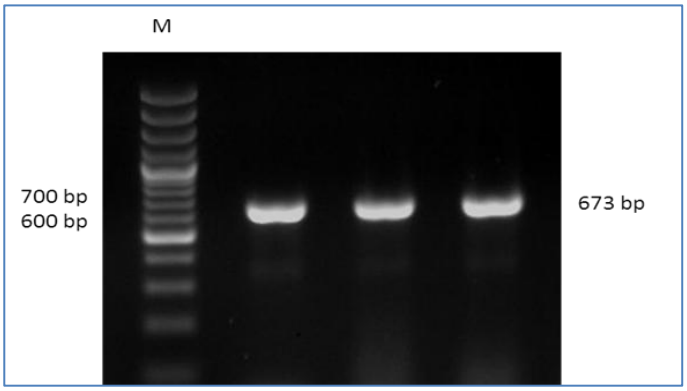

Fig-1: Amplification of GH gene in the studied duck populations. M: 100 bp ladder

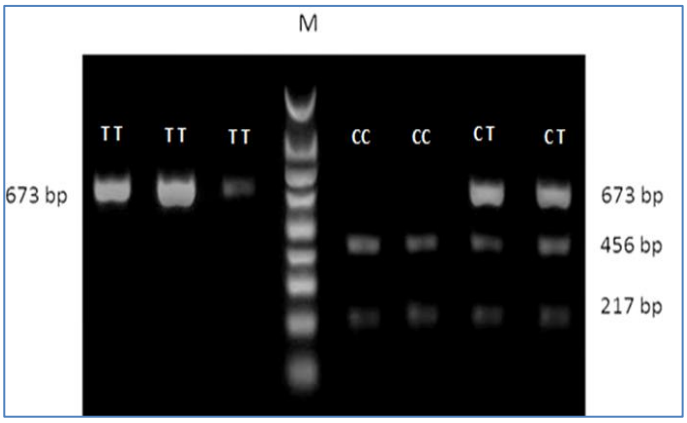

Fig-2: Restriction fragment patterns of GH gene after digesting with BsmFI. M: 100 bp ladder.

Table 1 lists the allele and genotype frequencies of the *GH* gene intron 2 in the three duck populations. The results indicated that *GHT* is the dominant allele, with frequencies of 0.91, 0.71, and 0.84 for Muscovy, Pekin, and Mulard ducks, respectively. Also, the most frequent genotype in all the examined ducks was *GH/TT*. Pekin ducks displayed the highest degree of genetic polymorphism for the duck *GH* gene intron 2. The genotype frequencies of the *GH/BsmFI* locus in this duck population were not in Hardy–Weinberg equilibrium ($P = 0.01$). In contrast, only two genotypes—*GH/TT* and *GH/CT*—were detected in the Muscovy and Mulard ducks, with frequencies of 0.82 and 0.18, and 0.67 and 0.33, respectively.

Table-1: Genotypic and allelic frequencies of GH gene intron 2 in different duck populations

Population	Genotype frequencies						Allele		HWE	
	Observed			Expected						
	GH/TT	GH/CT	GH/CC	GH/TT	GH/CT	GH/CC	T	C	x^2Calc	*P*-value
Muscovy	33(0.82)	7(0.18)	0(0.00)	33.30(0.83)	6.38(0.16)	0.30(0.01)	0.91	0.09	0.37	0.54
Pekin	24(0.60)	9(0.22)	7(0.18)	20.30(0.51)	16.38(0.41)	3.30(0.08)	0.71	0.29	8.13	0.01
Mulard	27(0.67)	13(0.33)	0(0.00)	28.05(0.70)	10.88(0.27)	1.05(0.03)	0.84	0.16	1.51	0.21

x^2 (HWE): Hardy–Weinberg equilibrium x^2value.

Association of GH with selected economic traits

Table 2 summarizes the results of the GLM analysis of associations between the GH RFLP polymorphisms and selected economic traits in Muscovy ducks. The ducks with *GH/TT* genotype were significantly ($P < 0.001$) heavier than those with the *GH/CT* genotype, producing 843.5 kg more weight than the latter. The dressing, breast, and thigh weight percentages were higher in *GH/TT* ducks than in

GH/CT ducks, by 3.47%, 2.06%, and 1.41%, respectively ($P < 0.001$).

Pekin ducks displayed a highly significant association ($P < 0.001$) among its different genotypes and the evaluated biometric traits (Table 3). The BW of *GH/TT* ducks was 3592.4 kg, which was higher than that of *GH/CT* ducks by 520.1 kg and *GH/CC* ducks by 699.3 kg. There was no significant difference between *GH/CT* and *GH/CC* ducks. Moreover, *GH/TT* genotype ducks had the highest dressing and breast weight percentages (74.15% and 51.47%, respectively) in comparison with the *GH/TC* ducks (71.96% and 49.56%, respectively) and the *GH/CC* ducks (70.19% and 48.13%, respectively). No associations were observed among genotypes with respect to the thigh weight.

Mulard ducks also exhibited significant association ($P < 0.001$) between the different *GH* genotypes and the biometric traits. The BW of *GH/TT* ducks was notably higher than that of *GH/CT* ducks by 332.8 kg (Table 4). The dressing and breast weight percentages of *GH/TT* ducks were higher than that of *GH/TC* ducks by 2.46% and 2.21%, respectively. No significant difference was noticed among the three genotypes for thigh weight. Tables 2, 3, and 4 show the allelic substitution and dominance effects of different genotypes.

Table-2: Least squares means (SE) of selected economic traits in different genotypes of GH gene of Muscovy ducks

Traits	Genotype		*P*-value	Additive gene effect	*P*-value	Dominance effect	*P*-value
	GH/TT	GH/CT					
Body weight (g)	4057.8 (31.63)[a]	3214.3 (10.69)[b]	<0.001	- 134.85(26.98)	0.042	156.95(33.65)	0.035
Dressing %	77.31 (0.11)[a]	73.84 (0.07)[b]	<0.001	- 3.22(0.09)	0.007	- 3.75(0.13)	0.001
Breast %	52.75 (0.09)[a]	50.69 (0.15)[b]	<0.001	- 2.82(0.13)	0.067	0.907(0.18)	0.035
Thigh %	24.58 (0.07)[a]	23.17 (0.04)[b]	<0.001	0.254 (0.09)	0.293	0.531 (0.11)	0.194

SE: standard error.
Values within a row with different superscripts differ significantly.

Table-3: Least squares means (SE) of selected economic traits in different genotypes of GH gene of Pekin ducks

Traits	Genotype			*P*-value	Additive gene effect	*P*-value	Dominance effect	*P*-value
	GH/TT	GH/CT	GH/CC					
Body weight (g)	3592.4 (21.57)[a]	3072.3 (32.45)[b]	2893.1 (17.65)[b]	<0.001	-115.69(29.14)	0.051	129.95(21.27)	0.039
Dressing %	74.15 (0.13)[a]	71.96 (0.08)[b]	70.19 (0.11)[c]	<0.001	- 0.907(0.12)	0.070	1.74(0.15)	0.000
Breast %	51.47 (0.08)[a]	49.56 (0.16)[b]	48.13 (0.04)[c]	<0.001	1.16(0.08)	0.198	1.10(0.11)	0.224
Thigh %	22.64 (0.20)	22.29 (0.31)	22.05 (0.21)	0.289	- 0.539(0.25)	0.728	- 0.907(0.19)	0.903

SE: standard error.
Values within a row with different superscripts differ significantly.

Table-4: Least squares means (SE) of selected economic traits in different genotypes of GH gene of Mulard ducks

Traits	Genotype		*P*-value	Additive gene effect	*P*-value	Dominance effect	*P*-value
	GH/TT	GH/CT					
Body weight (g)	3627.0 (18.94)[a]	3294.2 (36.47)[b]	<0.001	- 128.36(27.15)	0.048	141.08(32.88)	0.061
Dressing %	74.63 (0.13)[a]	72.17 (0.11)[b]	<0.001	- 2.56(0.09)	0.029	2.74(0.12)	0.009
Breast %	51.39 (0.08)[a]	49.18 (0.11)[b]	<0.001	- 1.25(0.07)	0.147	- 1.84(0.11)	0.199
Thigh %	23. 22 (0.15)	22.98 (0.21)	0.124	- 0.698(0.16)	0.506	0.439(0.19)	0.733

SE: standard error.
Values within a row with different superscripts differ significantly.

DISCUSSION

Previous reports confirm that the exons and 5′ regulatory regions of the chicken *GH* gene are highly conserved, and mutations predominantly occur in the introns [28-30]. Hence, the expression of *cGH* may be regulated by the introns or the 3′ untranslated region. In the current study, we inspected the polymorphisms in intron 2 of the duck *GH* gene. We identified three genotypes—*GH/TT*, *GH/CT*, and *GH/CC*—using the PCR–RFLP technique, with the genotype *GH/TT* being the most frequent. Pekin duck displayed the highest degree of genetic polymorphism, consisting of all the

three genotypes; contrarily, Mulard and Muscovy ducks showed only two genotypes—*GH/TT* and *GH/CT*. These findings are supported by the results of Mazurowski *et al*. [31]. However, Wu *et al*. [2] reported that the *BsmF1* restriction enzyme enabled the detection of the three genotypes in all the populations of ducks. This disparity may be a result of the difference in breeds used in the experiments.

As a single polypeptide chain protein, the growth hormone (GH) was formerly supposed to be participated only in the regulation of animal growth and development [32]. In the current study, we identified associations between the duck *GH* genotypes and biometric traits of body, dressing, and breast muscle weights, which are of economic importance. We conclude that ducks with *GH/TT* genotype display higher values of these traits compared to ducks of other genetic groups. Considering that the *GHT* allele had a beneficial effect on growth and carcass traits, it would be favorable to select this allele during breeding to improve these economically important traits in ducks. Similarly, Xu *et al*. [32] reported that SNPs in intron 2 and 3 of *GH* might impart some productive traits in duck. Mazurowski *et al*. [31] reported that Pekin and Mulard ducks with *GH/TT* genotype were superior to birds with *GH/CT* and *GH/CC* genotypes with respect to body weight, length of the breastbone, and length of the shank. However, our findings regarding Muscovy ducks contrast with those published by Wu *et al*. [2]. They reported that Muscovy ducks with the genotype *GH/CT* were heavier birds than those with other genotypes.

CONCLUSION

PCR–RFLP is an extremely useful molecular technique that detects variations in gene loci. Our study revealed three different genotypes in duck *GH* gene intron 2—*GH/TT*, *GH/TC*, and *GH/CC*. We also observed strong associations between the polymorphisms in the duck *GH* gene and various economic traits, including body, dressing, and breast weights. This indicated that the *GH* gene might be a candidate marker for enhancing economic traits in ducks. Further studies are required with other loci in the same gene regions or other genes to develop the best selection methods, based on genes linked with production traits in ducks.

Conflict of interest

The authors declare that they have no conflict of interest.

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