World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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# Genetic correlations in rabbit growth traits: Insights into the growth hormone receptor gene across diverse strains

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World Journal of Advanced Research and Reviews, 2024, 22(03), 1395–1406

Publication history: Received on 27 April 2024; revised on 18 June 2024; accepted on 21 June 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.3.1722

## Abstract

This study was carried out to investigate genetic correlations among growth traits and their association with the growth hormone receptor gene (GHR) in three rabbit strains. 150 weaner rabbits of three different strains comprising 50 New Zealand white (25 males and 25 females), 50 Dutch (25 males and 25 females) and 50 Hylamax (25 males and 25 females) were used. The experiment which lasted for 30 weeks, evaluated genetic correlation among growth traits and how it associated with GHR gene. Over the course of 30 weeks, we meticulously scrutinized the interplay of growth traits such as Chest Girth, Body length, Ear Length, Body weight, Abdominal circumference, Thigh Length, and Tail Length, seeking to uncover hidden genetic correlations lurking within. Employing cutting-edge techniques including Polymerase Chain Reaction and multivariate analysis via IBM SPSS, we dissected the data with precision. Our findings unveiled a captivating narrative of strain, sex, and age effects on body weight, with New Zealand White rabbits having highest values in all the parameters studied and different ages. But the intrigue didn't end there! By targeting the elusive GANTC restriction site with the formidable Hinf1 endonuclease, we uncovered a striking divergence among genotypes, shedding light on the profound impact of genetic variation on growth traits. Across the ages, from the tender weeks of 6 to the age of 30, our study revealed that significant disparities and subtle distinctions, painting a vivid portrait of genetic influence on rabbit growth. There were significant ($P<0.05$) disparities in all three rabbit strains across the various ages (6, 14, 18, 22, 26 and 30) respectively except for week 10 that showed no significant difference even though numerical variation existed. For week 6, the strains exhibited variations for CC, GC and GG genotypes respectively with Hylamax having significantly ($P<0.05$) higher value for GG and lowest for GC. New Zealand White and Dutch strains had similar values for GC but varied for GG. BL had a positive phenotypic correlation with LBW and EL at ($P<0.05$) level. Furthermore, TAL was highly substantial ($P<0.01$) with EL (0.01) and moderate in TL (0.02) at (0.01 and 0.05 levels). CG was positively correlated to BL but damagingly correlated to LBW.

**Keywords:** Genetic correlations; Rabbit; Growth hormone; Growth Traits; New Zealand; Allele.

## 1. Introduction

The domestic rabbit (*Oryctolagus cuniculus*) has been associated with man and has contributed to his well-being for many centuries. Rabbits are small mammals in the family Leporidae of the order Lagomorpha. There are about 305 strains of domestic rabbit. Best known for being prolific, rabbits are also herbivores which efficiently convert fodder to food (El-Sabrout and Aggag, 2017). According to McNitt *et al.* (2010), the only factor that has been found to be restricting rabbit growth and productivity in tropical and arid settings is calorie stress brought on by high ambient temperature. According to Aduku and Olukosi (2010), rabbit meat is an inexpensive meat source that has a high protein content and a low fat and cholesterol content. Rabbits are similar to domestic chickens in that they are highly prolific, have short

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gestation and generation intervals, are not taboo to produce or consume, and can survive on household trash and succulent leaves. They also have the ability to provide high-quality animal protein. Because they can subsist on forages and agricultural byproducts that humans do not consume, rabbits can easily be raised on a small or big scale in the tropics, where there is fierce rivalry between humans and animals for grains and legumes.

Due to its high protein level, low fat and cholesterol content, and overall healthful status, rabbit meat is regarded as a delicacy (Dalle Zotte, 2010). Other qualities of rabbits that may be helpful to a subsistence farming system are their tiny size and short generation interval, with an average gestation period of 30-31 days. They require a small amount of land and have a high growth rate, high feed efficiency, early marketing age, (Ortiz-Hernandez and Rubio-Luzano, 2011). It is used as a food, for fur, as an experimental subject in research, and as a source of much pleasure as a pet and fancy animal in some countries. Since rabbits have smaller bodies, shorter gestation periods, high production potential, quick growth rates, and can use forage and by-products as main components of their diet, the number of rabbit farms is growing in most developing nations (Cheeke, 2016).

Growth hormone is very important for growth in humans and animals as well, when growth hormone binds to growth hormone receptor (GHR), it causes receptor-dimerization which promotes cell growth and increases blood glucose and fatty acids levels (Zhang *et al*. 2009). Through the activation of tyrosinase kinase or the induction of insulin-like growth factor 1, GHR either directly or indirectly influences growth hormone (Brooks and Waters, 2010). The 11th chromosome of the rabbit genome houses the sequenced GHR gene, which has 10 exons and is annotated to 17 domains. The transcript length of 4024 bp translates to a protein length of 638 amino acids. Single Nucleotide Polymorphisms (SNP) can be detected by several ways. A novel technique for SNP detection and DNA sequence analysis was presented by Ota et al. (2007) utilizing the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, where the PCR products are incubated with restriction endonuclease to detect point mutations based on the fragmentation of the PCR products. The association of single nucleotide polymorphisms (SNPs) with different economical traits in rabbits has been successfully explored (Kotsyubenko *et al.*, 2017; Wu *et al.*, 2015). Deng et al. (2008) used PCR-SSCP to examine the polymorphism of the GHR gene and found two mutations (C705T and C810T) in five different populations of rabbits. By sequencing the exons and non-coding area of the GHR gene in rabbits, Fontanesi et al. (2016) investigated the polymorphism of the gene and discovered 10 SNPs, one of which was a missense mutation (g.63453192C>G or c.106C>G).

Understanding reproduction efficiency and genetic parameters like heritability and the genetic associations of significant economic traits are among the prerequisites for genetic improvement. Regretfully, the literature that is currently available for rabbits raised in tropical climates provides very little information on these parameters (Akanno and Ibe, 2015). Despite earlier initiatives in Nigeria for genetic improvement programs, these have been largely unsustainable because these were based on specific crosses (two or three-way crosses) among the most common commercial meat rabbit breeds which include New Zealand White, California, Chinchilla and Flemish Giant under on-station conditions. Many developing nations use heterogeneous or composite populations of rabbits, which are either the result of crosses between exotic breeds and local breeds or between breeds of exotic rabbits.

Molecular genetics offered new approaches for the genetic improvement of animals (Abdullah *et al.*, 2003). Such approaches accelerate the improvement steps and increase the genetic gain. One of these methods is the candidate gene strategy, which is applied in genetic selection programs and involves screening genes with known functions for polymorphism and connecting them to desired traits. Growth hormone is crucial for both human and animal growth. It does this by causing receptor-dimerization when it binds to the growth hormone receptor (GHR), which in turn stimulates cell growth and raises levels of fatty acids and blood sugar. The genetic parameters such as genetic correlation are often used as the basis for selection schemes and therefore, the genetic parameters should be estimated in each population (Akpan 2000; Odubote and Somade 1992).

When creating simple designs, the covariances between an individual's performance on one trait and that of their relatives on another are calculated, either directly or scaled as correlations or regressions. For instance, 2cov(X,Y) is an estimate of the (additive) genetic covariance, covA, and $\sqrt{\{[cov(X, Y)cov(Y, X)]/[cov(X, X)cov(Y,Y)]\}}$ is an estimate of the genetic correlation, rA, if cov(X,Y) is the sample covariance between trait X on the parent and trait Y on the offspring. The estimation of the genetic association usually has a significant standard error unless the data set is substantial. The correlated response to selection can also be used to estimate the genetic correlation, also known as "realized genetic correlation," in the event that one line out of a pair is selected for X and the other line for Y.

## 2. Materials and methods

### 2.1. Study Location

This study was carried out at the Rabbitry Unit of the Teaching and Research farm of Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers State. Port Harcourt lies between longitude $6^0$ 59' 54"E and latitude $4^0$ 47' 21"N with average monthly temperature and relative humidity of 22.54 – 31.03$^0$C and 69.08 – 112.47% respectively. The average rainfall in Port Harcourt is 200.45mm (Uko and Tamunobereton-Ari, 2013).

### 2.2. Experimental Animals

The study was conducted with a total of 150 weaned rabbits belonging to three strains as follows: New Zealand White (25 males and 25 females), Dutch (25 males and 25 females) and Hylamax (25 males and 25 females), respectively. The rabbits were obtained from O. G farms Ogbomosho Oyo State, Lautech Teaching and Research farm Ogbomosho Oyo State and O. G. Farms, Egbeda Ibadan respectively. These were introduced into the Rabbitry Unit of the Teaching and Research Farm of Rivers State University, Port Harcourt, Rivers State. The experimental animals were randomly allocated into separate hutches according to sex and strain. They were given two weeks to get used to their new surroundings. This was carried out to guarantee precise data collection on an individual basis. The rabbits were fed commercial grower food (Rainbow grower mash) with 16.5% CP and 2500ME kcal/kg on an ad libitum basis. They were periodically vaccinated and also given multivitamins and antibiotics via drinking water as the need arose.

### 2.3. Measurements and Data Collection

Measurements were taken on growth traits and blood for molecular analysis to determine genetic correlation and its association with growth hormone receptor genes.

#### *2.3.1. Blood sample collection and DNA extraction*

Samples were gotten from ear vein in sterile tube containing EDTA as anticoagulant. The samples were mixed well and stored at -20 °C until use. The blood was collected from a total of 30 rabbits from the 3 strains, that is 10 (i.e., 5 males and 5 females) respectively per strain. The three highest and the two lowest body weights of each strain for male and female respectively were taken for DNA sequence analysis and their weights were recorded.

#### *2.3.2. DNA Extraction*

Using the Zyno Research, USA Quick-DNA Miniprep kit, DNA was extracted from each rabbit using the following manufacturing protocol: fill a microcentrifuge tube with up to 100 ml of sample, add 10 ml of Bio'fluid and cell buffer (red), and 20 ml of proteinase K. Mix well, and incubate the tube at 55 oC for ten minutes. Add 220 volume genomic Binding Buffer to the digested sample and mix thoroughly; Transfer the mixture to a Zymo-SpinTm 11C-XL column in a collection tube. Centrifuge (>12,000Xg) for 1minute. Throw away the collection tube containing the flow through. Fill a new collection tube with 200 ml of DNA Pre-wash Buffer, add it to the column, and centrifuge for one minute.

#### *2.3.3. DNA Quantification/Quality Check*

Using gel electrophoresis and a portable gel hood with a built-in blue LED (470 nm) from Royal Biotech/Biolympics (www.royalbiotech.com), the quality of the DNA and the PCR products was evaluated for about an hour at a constant voltage of 100 volts in 1X TBE. They were visualized by ethidium bromide staining and photographed under ultraviolet light. The ladder used is 100 base pair Ladder from Thermo Scientific.

#### *2.3.4. Polymerase Chain Reaction*

The following cocktail mix and conditions were applied to the DNA for the 25µl reaction. Polymerase Chain Reaction: 2.5 µl of 10xPCR buffer, 1.0µl of 25mn $MgCl_2$, 1.0µl of 5pMol forward primer (Table 3.1) and 1.0µl of Taq 5u/µl, 3.0 µl of 10mg/µl DNA and 13.4µl water. A touch down PCR condition was used which involved initial denaturation at 94$^o$C for 5 minutes, 9 cycles of denaturation at 94$^o$C for 15 seconds, Annealing temperature at 65$^o$C at 20 seconds and extension at 72$^o$C for 30 seconds. This was followed by 35 cycles of denaturation at 94$^o$C for 15 seconds, annealing temperature at 55$^o$C at 20 seconds and extension at 72$^o$C for 30 seconds and a final extension at 72$^o$C for 7 minutes.

**Table 1** PCR primers used to amplify growth hormone receptor gene in rabbits

| Primer Name | Primer sequence | Product | Annealing | Location |
|---|---|---|---|---|
| GHR Forward | 5" – TCC GGGGGTACGGGGTCATTAGGTT – 3" | 336bp | 56 | Exon 6 |
| GHR Reverse | 5" – AGAGGGGTTGCTGGGGTAGGGG – 3" | | | |

Source: (Zhang *et al.* 2012).

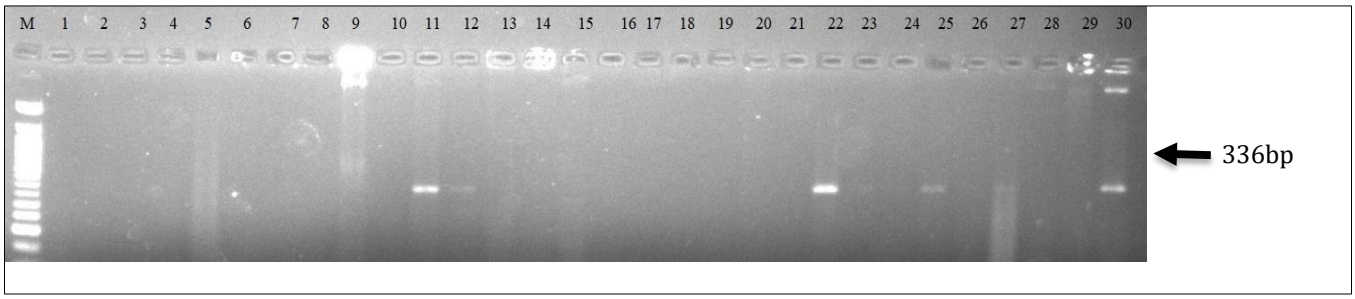


**Figure 1** Gel electrophoresis picture showing amplified PCR products (336bp) of growth hormone receptor gene in rabbits

### 2.4. Sequence Analysis

Sequences were edited and aligned using Molecular Evolutionary Genetics Analysis (MEGA) Version 11. Using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), we were able to confirm our sequence and see which sequences in rabbits were providing meaningful alignments with the growth hormone receptor gene. Genetic distance between rabbit strains, Nucleotide sequence alignment showing conserved regions and mutation as well as a dendogram showing the phylogenic relationship between New Zealand White, Dutch and Hylamax Rabbits used in this study and others from gene bank in growth hormone receptor gene was conducted using MEGA 11 (Kumar, 2013).

In addition, Genetic polymorphism, Genetic Differentiation Estimates, Gene Flow Estimates and Haplotype Distribution in growth hormone receptor gene in rabbits were all determined DNASp Version 5 (Librado and Rozas, 2009).

Restriction Fragment Length Polymorphisms (RFLPs): MSP1 was used to digest the amplified DNA fragments of the growth hormone receptor gene. A reaction volume of 20 µl was used for the RFLP, which included 7 µl H2O, 2 µl buffer, 10 µl PCR product, and 1 µl restriction enzyme (Zhou et al., 2005).

Sequencing and analysis of the GHR gene: The amplified PCR products of exon 1 of GHR was sent to International Institute for Tropical Agriculture (IITA) Ibadan for sequence using ABI 3730XL DNA sequencer (Applied Biosystem, USA). Ten samples from each strain was sent for sequencing (5 males and 5 females from each strain). The sequence results was analyzed using Chromas 1.45. Sequence comparisons were performed using the BLAST program from the National Center for Biotechnology information. The sequences were aligned using CLUSTAL-W version 1.8 (Thompson *et al.* 1994). A neighbor joining tree was constructed using MEGA version 6 (Tamura *et al.* 2011). Sequence translation was performed using MEGA version 6. SNP detector: A Software Tool for Sensitive and Accurate SNP Detection (Zhang et al., 2005) was used to identify and evaluate single nucleotide polymorphisms (SNPs).

### 2.5. Experimental Design and Data Analysis

The study was conducted using a 3 x 2 factorial in a completely randomized design in order to evaluate the growth parameters. Accordingly, the data was subjected to a multivariate analysis outlined in the General Linear model using IBM SPSS (Version 22, 2013). Strain type (NZW, DUT and HYL) and Sex type (male and female) as main effects. Interactions between the main effects were also analyzed. Significant means at $P<0.05$ were separated using the Turkey test technique. The linear model under the stated design for the study on the growth parameters were as follows:

$Xijk = \mu + Ai + Sj + ASij + eijk$

Where

Xijk = observation made on each trait evaluated
μ = the overall population mean
Ai = effect of the ith strain on the observed trait (*i*=1, 2, 3)
Sj = effect of the jth sex on the measured trait (j=1, 2)
ASij = effect of the interaction between strain and sex on the measured trait
eijk = random error.

### 2.6. Molecular analysis

The experimental configuration for the molecular studies was a 3 x 2 x 7 factorial in a randomized complete block design, with the 4-weekly factor serving as the block. The General Linear Model (GLM) procedure of SPSS was utilized to examine or evaluate the relationship between the body weights and the SNP (c.106C>G). The appropriate statistical model was:

$$Yijkl = \mu + Bi + Sj + Ak + eijkl$$

where, Y is the dependent variable under study,

μ is the overall mean,

B, S and A indicate the fixed effects of breed (3 levels), sex (2 levels) and 4-weekly age (7 levels), respectively.

### 2.7. Genotypic and allelic frequencies of the three rabbit strains

Table 1 displays the genotypic and allele frequencies of the three rabbit strains. As the research focused on sexual dimorphisms, genetic correlations between growth traits and their relationship to the growth hormone receptor gene in three strains of rabbits, the GANTC restriction site—which is present twice in allele C and once in allele G—was targeted using the restriction endonuclease Hinf1. As a result, three fragments (210, 162, and 107 bp) for allele C and only two fragments (317 and 162 bp) for allele G are produced when the PCR fragment (479 bp) is incubated with Hinf1. Then, using the generated banding pattern for each individual, the genotypes of the animals can be identified directly; for the CC genotype, this results in two bands appearing (317 and 162 bp), for the GG genotype, three bands (210, 162 and 107 bp), and for the heterozygous CG genotype, four bands (317, 210, 162 and 107 bp). Table (4.2) displays the genotypic frequencies of the three strains of rabbits. It is noteworthy that the female New Zealand White, Dutch, and Hylamax rabbits did not exhibit the genotype CC, which was unexpected. Similarly, the female Dutch rabbits did not exhibit the heterozygous genotype. Across all individuals of the three strains, the frequency of the CC genotype was found to be as low as 0.10 overall. Conversely, the heterozygous individuals had a frequency of 0.25, but the total frequency of the GG genotype was 0.65. In comparison to NWZ (0.50) and Hylamax (0.50) rabbits, the frequency of the GG genotype was higher in Dutch (0.80) rabbits.

**Table 1** Genotypic and allelic frequencies of the three rabbit strains

| **Rabbit strains** | **N** | **Genotype frequency** | | | **Allele frequency** | |
|---|---|---|---|---|---|---|
| | | CC | CG | GG | C | G |
| All individual | 150 | 0.10 | 0.25 | 0.65 | 0.225 | 0.776 |
| New Zealand White | 50 | 0.10 | 0.30 | 0.50 | 0.425 | 0.874 |
| Dutch | 50 | 0.10 | 0.10 | 0.80 | 0.150 | 0.851 |
| Hylamax | 50 | 0.10 | 0.40 | 0.50 | 0.300 | 0.700 |
| New Zealand White(M) | 25 | 0.20 | 0.20 | 0.60 | 0.300 | 0.700 |
| New Zealand White(F) | 25 | 0.00 | 0.00 | 1.00 | 0.000 | 1.000 |
| Dutch(M) | 25 | 0.20 | 0.40 | 0.40 | 0.400 | 0.600 |
| Dutch(F) | 25 | 0.00 | 0.40 | 0.60 | 0.200 | 0.800 |
| Hylamax(M) | 25 | 0.20 | 0.00 | 1.00 | 0.000 | 1.000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hylamax(F)) | 25 | 0.00 | 0.40 | 0.40 | 0.400 | 0.600 |

### 2.8. Association analysis of GHR genotypes with body weight (g) at different ages in three strains of Rabbits

The associations between genotype and body weight over age are presented in Table (2). The effect of sex was not considered for association analysis not only because of the insignificance of sex effect in most ages, but also because the CC genotype was not detected in the females. Upon analyzing all 150 rabbits collectively, it was found that at 6, 10, 14, 18, 22, 26, and 30 weeks of age, there was a highly significant ($P<0.001$) difference between the CC genotype and the other two genotypes. These high levels of significance indicate that the differences between individuals were attributed to the genotype effect. Nonetheless, insignificant disparities were found between CG and GG genotypes. Similar results were obtained when each strain was analyzed separately. There were no significant variances between the genotypes GG and GC except for Dutch rabbit at 30 weeks of age. The difference between the GC and GG genotypes was clearly unaffected by the heterozygous allele C presence, despite the notable increases in body weight observed in the homozygous form of the allele.

**Table 2** Association analysis of GHR genotypes with body weight (g) at various ages in three strains of Rabbits. (Mean ±SEM)

| **Weeks** | **Strains** | **Genotype** | | | **P value** |
|---|---|---|---|---|---|
| | | CC | GC | GG | |
| BW6 | All individuals | 933.8±65.9$^{a}$ | 657.4±43.9$^{b}$ | 575.7±49.8$^{c}$ | 0.001 |
| | New Zealand | 945.0±23.3$^{a}$ | 779.3±20.2$^{b}$ | 749.3±23.3$^{b}$ | 0.001 |
| | Dutch | 909.5±50.2$^{a}$ | 560.0±22.5$^{b}$ | 445.5±25.1$^{c}$ | 0.000 |
| | Hylamax | 913.6±40.2$^{a}$ | 511.0±18.5$^{b}$ | 424.5±27.1$^{c}$ | 0.001 |
| BW10 | All individuals | 1155.6±93.4 | 1216.7±71.8 | 966.0±98.7 | 0.192 |
| | New Zealand | 958.9±62.3 | 1127.8±62.2 | 823.8±44.1 | 0.601 |
| | Dutch | 948.4±70.6 | 1125.7±71.8 | 815.5±49.3 | 0.417 |
| | Hylamax | 921.4±65.8 | 1015.7±45.4 | 804.5±30.3 | 0.510 |
| BW14 | All individuals | 1836.1±97.6$^{a}$ | 1397.1±65.1$^{b}$ | 1344.4±73.8$^{b}$ | 0.002 |
| | New Zealand | 1868.1±83.3$^{a}$ | 1552.5±72.1$^{b}$ | 1495.0±83.3$^{b}$ | 0.030 |
| | Dutch | 1740.0±167.9$^{a}$ | 1272.8±75.1$^{b}$ | 1231.0±83.9$^{c}$ | 0.045 |
| | Hylamax | 1855.5±103.9$^{a}$ | 1804.8±71.1$^{b}$ | 1305.0±84.1$^{c}$ | 0.030 |
| BW18 | All individuals | 2351.2±148.6$^{a}$ | 1906.3±99.0$^{b}$ | 1590.7±112.3$^{c}$ | 0.002 |
| | New Zealand | 2514.4±116.9$^{a}$ | 2131.3±101.3$^{b}$ | 1870.0±116.9$^{c}$ | 0.017 |
| | Dutch | 1862.2±131.3$^{a}$ | 1726.4±58.7$^{b}$ | 1381.3±65.6$^{c}$ | 0.009 |
| | Hylamax | 2245.1±125.3$^{a}$ | 2026.4±65.7$^{b}$ | 1981.3±71.2$^{b}$ | 0.007 |
| BW22 | All individuals | 2751.2±148.6$^{a}$ | 2306.3±99.0$^{b}$ | 1990.7±112.3$^{c}$ | 0.002 |
| | New Zealand | 2914.4±116.9$^{a}$ | 2531.3±101.3$^{b}$ | 2270.0±116.9$^{c}$ | 0.017 |
| | Dutch | 2262.1±131.3$^{a}$ | 2126.4±58.7$^{b}$ | 1781.3±65.6$^{c}$ | 0.009 |
| | Hylamax | 2414.5±122.9$^{a}$ | 2611.3±107.1$^{b}$ | 2470.0±131.9$^{c}$ | 0.016 |
| BW26 | All individuals | 3551.5±148.6$^{a}$ | 2706.3±99.0$^{b}$ | 2790.7±112.3$^{b}$ | 0.002 |
| | New Zealand | 3314.3±116.9$^{a}$ | 2731.3±101.3$^{b}$ | 2970.0±116.9$^{b}$ | 0.017 |
| | Dutch | 2462.1±131.3$^{b}$ | 2526.4±58.7$^{a}$ | 2581.3±65.6$^{a}$ | 0.009 |

| | | | | | |
|---|---|---|---|---|---|
| | Hylamax | 3018.4±104.9[b] | 3631.3±109.2[a] | 3070.0±117.1[b] | 0.016 |
| BW30 | All individuals | 3851.4±148.6[a] | 3106.3±99.0[b] | 3190.7±112.3[b] | 0.002 |
| | New Zealand | 3214.4±116.9[a] | 3131.3±101.3[b] | 3370.0±116.9[a] | 0.017 |
| | Dutch | 2862.4±131.3[b] | 2926.4±58.7[a] | 3081.3±65.6[a] | 0.009 |
| | Hylamax | 3111.3±122.1[b] | 3037.3b±107.3 | 3175.1±1144.9[a] | 0.016 |

Means with different superscript on the same row are significantly different (P<0.05).

### 2.9. Association analysis of GHR genotypes with body length (cm) at different ages in three strains of Rabbits

The associations between genotype and body length are indicated in Table (3). The difference between the CC genotype and the other two genotypes at 6, 10, 14, 18, 22, 26, and 30 weeks of age was extremely significant (P<0.001) when all 150 rabbits were examined collectively. Although Dutch strain showed no significant variation in week 14, however numerical differences existed. These high levels of significance indicate that the differences between individuals were attributed to the genotype effect. There were significant (P<0.05) variances between the genotypes GG and GC except for Dutch rabbit at 10 and 14 of age and Hylamax at week 10 of age. It was clear that the presence of allele C in the heterozygous genotype did not affect the difference between GC and GG genotypes, although the considerable increases in body length when allele C was presented in the homozygous form.

**Table 3** Association analysis of GHR genotypes with body length (BL) at different ages in the three strains of rabbits. (Mean ±SEM)

| **Weeks** | **Strains** | **Genotype** | | | **P value** |
|---|---|---|---|---|---|
| | | CC | GC | GG | |
| BL6 | All individuals | 28.8±65.9[a] | 27.4±43.9[b] | 27.7±11.8[b] | 0.001 |
| | New Zealand | 27.0±13.3[b] | 27.3±20.2[b] | 28.3±11.3[a] | 0.009 |
| | Dutch | 28.0±10.5[a] | 26.0±22.5[c] | 27.5±15.1[b] | 0.001 |
| | Hylamax | 28.0±20.7[a] | 28.0±18.5[a] | 26.5±17.1[b] | 0.001 |
| BL10 | All individuals | 29.1±93.5 | 27.7±71.8 | 28.0±98.7 | 0.122 |
| | New Zealand | 28.9±62.3[a] | 27.8±62.2[b] | 27.3±44.1[b] | 0.031 |
| | Dutch | 29.4±70.6 | 25.7±71.8 | 28.5±49.3 | 0.517 |
| | Hylamax | 29.4±65.8 | 26.7±45.4 | 28.6±30.3 | 0.711 |
| BW14 | All individuals | 37.0±97.6[a] | 37.1±65.1[a] | 34.4±73.8[b] | 0.002 |
| | New Zealand | 36.0±83.3[a] | 35.5±72.1[b] | 35.0±83.3[b] | 0.056 |
| | Dutch | 37.0±167.9 | 37.8±75.1 | 36.0±83.9 | 0.071 |
| | Hylamax | 36.0±103.9[a] | 36.8±71.1[a] | 35.0±84.1[b] | 0.050 |
| BL18 | All individuals | 38.3±148.6[a] | 36.3±11.0[b] | 36.7±112.3[b] | 0.003 |
| | New Zealand | 37.3±116.9[a] | 36.3±11.3[b] | 35.0±116.9[c] | 0.011 |
| | Dutch | 36.0±131.3[b] | 36.4±38.7[a] | 36.3±65.6[b] | 0.001 |
| | Hylamax | 37.0±125.3[a] | 36.4±25.7[b] | 36.3±71.2[b] | 0.003 |
| BL22 | All individuals | 41.3±148.6[a] | 39.3±99.0[b] | 38.7±112.3[c] | 0.002 |
| | New Zealand | 40.3±116.9[a] | 38.3±101.3[b] | 37.0±116.9[c] | 0.017 |
| | Dutch | 41.0±131.3[a] | 39.4±58.7[b] | 37.3±65.6[c] | 0.009 |
| | Hylamax | 41.3±122.9[a] | 37.3±107.1[b] | 37.0±131.9[b] | 0.016 |
| BL26 | All individuals | 42.3±148.6[a] | 42.3±99.0[a] | 41.7±112.3[b] | 0.002 |

| | | | | | |
|---|---|---|---|---|---|
| | New Zealand | 44.3±116.9[a] | 41.3±101.3[b] | 40.0±116.9[c] | 0.017 |
| | Dutch | 46.2±131.3[a] | 42.6±58.7[b] | 41.3±65.6[c] | 0.002 |
| | Hylamax | 41.3±104.9 | 41.3±109.2 | 41.0±117.1 | 0.015 |
| BL30 | All individuals | 44.3±148.6[a] | 42.3±99.0[b] | 44.7±112.3[a] | 0.004 |
| | New Zealand | 44.3±116.9[a] | 41.3±101.3[c] | 43.0±116.9[b] | 0.011 |
| | Dutch | 46.0±131.3[a] | 41.4±58.7[c] | 42.3±65.6[b] | 0.005 |
| | Hylamax | 45.3±122.1[a] | 41.3±107.3[c] | 43.0±114.4[b] | 0.016 |

Means with different superscript on the same row are significantly different (P<0.05).

### 2.10. Association analysis of GHR genotypes with Ear length (EL) at different ages in rabbits.

The association analysis of Growth Hormone Receptor (GHR) gene with ear length at different ages in the three strains of rabbits are presented in Table 4. There were significant (P<0.05) disparities in all three rabbit strains across the various ages (6, 14, 18, 22, 26 and 30) respectively except for week 10 that showed no significant difference even though numerical variation existed.

For week 6, the strains exhibited variations for CC, GC and GG genotypes respectively with Hylamax having significantly (P<0.05) higher value for GG and lowest for GC. While their GG values varied, the GC values of the Dutch and New Zealand White strains were similar. The relevance of the CC genotype was the same for all three strains. The GG genotype values for week 30 were highest for New Zealand White and Dutch, while the CC genotype values were highest for Hylamax. The CC genotype tends to favour Hylamax more than the other strains studied. Ages 14, 18, 22, 26 respectively showed significant differences for the 3 strains for CC, GC and GG genotypes.

**Table 4** Association analysis of GHR genotypes with Ear length (EL) at different ages in the three strains of rabbits

| **Weeks** | **Strains** | **Genotype** | | | **P value** |
|---|---|---|---|---|---|
| | | CC | GC | GG | |
| EL6 | All individuals | 8.80±65.9[a] | 8.40±43.9[c] | 8.57±49.8[b] | 0.001 |
| | New Zealand | 8.35±23.3[b] | 8.90±20.2[a] | 8.13±23.3[c] | 0.001 |
| | Dutch | 8.14±50.2[b] | 8.60±22.5[a] | 8.15±25.1[b] | 0.003 |
| | Hylamax | 8.13±40.2[b] | 8.10±18.5[c] | 8.14±27.1[a] | 0.001 |
| EL10 | All individuals | 9.01±93.5 | 9.17±71.8 | 9.01±98.7 | 0.121 |
| | New Zealand | 9.48±62.3 | 9.07±62.2 | 9.08±44.1 | 0.201 |
| | Dutch | 9.18±70.6 | 9.15±71.8 | 9.05±49.3 | 0.314 |
| | Hylamax | 9.12±65.8 | 9.15±45.4 | 9.05±30.3 | 0.319 |
| EL14 | All individuals | 10.15±97.6[c] | 10.97±65.1[a] | 10.44±73.8[b] | 0.002 |
| | New Zealand | 10.68±83.3[b] | 10.52±72.1[c] | 10.95±83.3[a] | 0.013 |
| | Dutch | 10.40±67.9[b] | 10.72±75.1[a] | 10.31±83.9[c] | 0.013 |
| | Hylamax | 10.55±103.9[a] | 10.48±71.1[b] | 10.05±84.1[c] | 0.020 |
| EL18 | All individuals | 11.30±118.6[b] | 11.03±99.0[c] | 11.90±112.3[a] | 0.002 |
| | New Zealand | 11.43±116.9[a] | 11.31±101.3[b] | 11.07±116.9[c] | 0.010 |
| | Dutch | 11.62±131.3[a] | 11.26±58.7[c] | 11.36±65.6[b] | 0.092 |
| | Hylamax | 11.04±125.3[c] | 11.26±65.7[b] | 11.44±71.2[a] | 0.077 |
| EL22 | All individuals | 11.81±148.6[a] | 11.73±99.0[c] | 11.79±112.3[b] | 0.002 |
| | New Zealand | 11.84±116.9[b] | 11.93±101.3[a] | 11.70±116.9[c] | 0.017 |

| | | | | | |
|---|---|---|---|---|---|
| | Dutch | 11.92±131.3[a] | 11.47±58.7[c] | 11.88±65.6[b] | 0.009 |
| | Hylamax | 11.74±122.9[b] | 11.37±107.1[c] | 11.90±131.9[a] | 0.016 |
| EL26 | All individuals | 12.31±148.6[b] | 12.30±99.0[b] | 12.70±112.3[a] | 0.002 |
| | New Zealand | 12.81±116.9[a] | 12.31±101.3[b] | 12.05±116.9[c] | 0.014 |
| | Dutch | 12.46±131.3[a] | 12.42±58.7[b] | 12.35±65.6[c] | 0.004 |
| | Hylamax | 12.83±104.9[a] | 12.31±109.2[b] | 12.01±117.1[c] | 0.016 |
| EL30 | All individuals | 13.22±148.6[a] | 13.10±99.0[c] | 13.19±112.3[b] | 0.002 |
| | New Zealand | 13.12±116.9[c] | 13.19±101.3[b] | 13.24±116.9[a] | 0.017 |
| | Dutch | 13.08±131.3[b] | 13.01±58.7[c] | 12.90±65.6[a] | 0.009 |
| | Hylamax | 13.11±122.1[a] | 12.09±107.3[c] | 13.00±114.9[b] | 0.016 |

Means with different superscript on the same row are significantly different (P<0.05).

Table 5 presents the phenotypic correlation between body morphometrics using pooled facts of the three strains. BL had a positive phenotypic correlation with LBW and EL at (P<0.05) level. Furthermore, TAL was highly substantial (P<0.01) with EL (0.01) and moderate in TL (0.02) at (0.01 and 0.05 levels). CG was positively correlated to BL but damagingly correlated to LBW. For the three strains singly, strong positive phenotypic correlation was perceived between LBW, with TAL, TL and AC. This indicates that LBW, TAL, BL, TL and AC are strong aspects for predicting growth and body weight in rabbits.

**Table 5** The phenotypic correlation of New Zealand, Dutch, and Hylamax rabbit strains with respect to body weight and body morphometrics

| | **LBW** | **BL** | **EL** | **TAL** | **TL** | **CG** | **AC** |
|---|---|---|---|---|---|---|---|
| LBW | 1 | 0.621** | 0.437** | 0.581** | 0.568** | 0.765 | 0.753** |
| BL | | 1 | 0.426** | 0.616* | 0.703** | 0.541** | 0.652** |
| EL | | | 1 | 0.581** | 0.402** | 0.406** | 0.442** |
| TAL | | | | 1 | 0.646** | 0.495** | 0.583** |
| TL | | | | | 1 | 0.531** | 0.720** |
| CG | | | | | | 1 | 0.844** |
| AC | | | | | | | 1 |

**. The significance level for the correlation is 0.01 (2-tailed).LBW = live body weight, BDL = body length, EL = ear length, TAL = tail length, TL = Thigh length, CG = chest girth, AC = abdominal circumference.

## 3. Discussion

### 3.1. Genotypic and Allele Frequency and Polymorphism

Targeting the GANTC restriction site, which is present twice in allele C and once in allele G, the restriction endonuclease Hinf1 was employed in the study because it focused on sexual dimorphisms, genetic correlations between growth traits, and their relationship to the growth hormone receptor gene in three strains of rabbits. Consequently, allele C yields three fragments (210, 162, and 107 bp) upon incubation of the PCR fragment (479 bp) with Hinf1, whereas allele G yields only two fragments (317 and 162 bp). Then, based on the generated banding pattern for each individual, the genotypes of the animals can be identified directly; for the CC genotype, this results in two bands appearing (317 and 162 bp), for the GG genotype, three bands (210, 162 and 107 bp), and for the heterozygous CG genotype, four bands (317, 210, 162 and 107 bp). Table (1) displays the genotypic and allelic frequencies of the genotyped rabbit animals. It was unexpected that the females of the New Zealand White, Dutch, and Hylamax rabbit strains did not exhibit the genotype CC, nor did the females of the Dutch strain exhibit the heterozygous genotype. Overall, it was discovered that just 0.10 percent of the two breeds' total individuals carried the CC genotype. Conversely, the heterozygous population had a frequency of 0.25, although the total number of GG genotype individuals was 0.65. Compared to NWZ (0.50)

rabbits, the frequency of the GG genotype was higher in Dutch (0.80) rabbits. The males of all three strains had the same frequency (0.20) of the CC genotype. After genotyping 51 NWZ rabbits, Gencheva et al. (2017) found that the GG genotype was less common (0.392) than it was in the current study, while the heterozygous genotype (CG) frequency was similar at 0.529. Additionally, they disclosed the CC genotype's extremely low frequency of (0.078). For the C and G alleles, the frequencies of the two alleles were determined to be 0.225 and 0.775, respectively. The existence of the C allele in a Chinese rabbit population was found to be lower (0.323) than that of the G allele (0.677), which is consistent with the findings of Zhang et al. (2012). The identical mutation was genotyped using PCR-RFLP by Migdal et al. (2019); CC genotype frequencies varied from 0.03 to 0.47. The moderate frequency of the C allele (0.15 – 0.30) paired with the low frequencies of CC genotypes (≤0.10) could indicate the existence of some sort of natural selection working against the C allele.

### 3.2. Genotype Effect and Association with Body Weight

Table (2) displays the relationships between body weight and genotype across age. The sex effect was not taken into account for the association analysis due to the lack of significance of the sex effect at most ages as well as the absence of the CC genotype in the females. The CC genotype differed significantly ($p \leq 0.002$) from the other two genotypes at 6, 10, 14, 18, 22, 26, and 30 weeks of age when all 150 rabbits were examined collectively. These high levels of significance suggest that the genotype effect was responsible for the individual differences. Nevertheless, few differences were discovered between the CG and GG genotypes. When every breed was examined independently, comparable outcomes were attained. With the exception of the Dutch rabbit at 30 weeks of age, there were no appreciable variations between the genotypes GG and GC. Despite the noticeable increases in body weight when allele C was present in the homozygous form, it is evident that the heterozygous genotype's existence did not alter the difference between the GC and GG genotypes. Zhang et al. (2012) published similar results, showing that CC genotypes were linked to the highest values for both slaughter weight and 84-day weight. Within exon 10 of the GHR gene, Deng et al. (2008) discovered a substantial ($p \leq 0.05$) correlation between live weight, visceraste weight, and slaughter % in five different rabbit populations, and two GHR mutations (G.63537066C>T and G.63537228A>G). Additionally, g.63453192C>G or c.106C>G, a GHR gene mutation, was found by Fontanesi et al. (2016) and was significantly ($P < 0.05$) correlated with the body weight of rabbits after 70 days. Meat weight and carcass traits were linked to the same mutation (c.106C>G) (Migdal et al., 2019; Zhang et al., 2012). In the first exon of the GHR gene, a different mutation was discovered and linked to the growth performance of NWZ, V-line, and Alexandria rabbits (Sahwan et al., 2014). Conversely, a missense mutation in the GHR gene was found by El-Sabrout and Aggag (2017) in Alexandria and V-line rabbits, and it was unrelated to any economical attribute. The findings showed that whereas CC genotypes were less common than other genotypes, they had superior body weights. The GHR gene's c.106C>G mutation, which affects codon use, is a promising candidate gene to boost rabbit body weight selection.

### 3.3. Significant alignment in GHR genes in rabbits

The transmembrane protein known as the growth hormone receptor (GHR) has 620 amino acids. This receptor belongs to the family of Type I cytokine receptors. GHR can be found in two different forms: a soluble GH binding protein (GHBP) and a full-length membrane-bound receptor. Growth hormone receptors (GHRs) are present on the cell surfaces of numerous bodily tissues, such as the kidney, liver, muscle, and adipose tissue, as well as in the early stages of embryonic and fetal development. The marketing weight of the three rabbit strains exhibits a strong correlation with the identified SNPs. These findings concur with those of Zhang et al. (2012) and Fontanesi et al. (2012). The outcomes of marker-assisted selection proved successful in identifying rabbits with exceptional growth performance. Furthermore, this study's investigation revealed that the GHR gene may be a candidate gene for use in marker-assisted selection (MAS).

The following SNPs were found when the sequence data of 30 rabbits (Ten NZW, Ten Dutch, and Ten Hylamax) were aligned: At nucleotide No. 5 (G-T) in NZW 1, at 32 (C-A) in Dutch, at 52 (G-T) in NZW 1, at 55 (G-T) in NZW 1. At 70 (G-C) at Dutch, at 83 (C-T) in NZW 1, at 97 (A-G) in NZW 1, at 112 (T-G) in NZW 1. At 125 (A-C) NZW 1, at 137 (G-A) in NZW 1, at 138 (G-T) in NZW 1. at 143 (G-A) Dutch, at 144 (A-G) in NZW 1, at 148 (C-T) in NZW 1. At 149 (G-C) NZW 1. At 150 (G-C) in NZW 2, NZW 5, Hylamax 2 and Dutch. At 151 (A-G) in NZW 2, NZW 4, NZW 5, Hylamax 2 and Dutch 4. The percentage comparison and phylogenetic tree among thirty rabbits (Ten NZW, Ten Dutch, and Ten Hylamax.) based on sequence data showed the presence of highest similarity (100%) between NZW 3, Dutch 2, Dutch 5, Hylamax 1, 3, 4 and 5. and also 100% similarity between NZW 2, NZW 5, Dutch 1 and Hylamax 2. One hundred percent comparison was noticed also between NZW 4 and Dutch 4. New Zealand White 1 and Dutch 3 rabbits' sequence had least comparison with that of Hylamax rabbits as seen in table (4.13) and Fig (3 and 4). Again, 100 percent comparison was also detected between NZW 4 and Dutch 4. Because genetic correlations make the relationship between genotype performance in various environments clear, they are helpful in understanding genotype performance in different environments and in supporting the identification of G × E interactions (Sgrò and Blows, 2004). Genetic correlations, for instance, can indicate if adaptation to several stressors can happen concurrently in multistressor research (Clark et al., 2013; Foo et al., 2012,

2014; Sgrò and Blows, 2004). Understanding evolutionary processes requires an understanding of genetic correlations, which are defined as the percentage of variation shared by two genetic features (Astles et al., 2006). When a trait is expressed in two or more contexts, it is seen as having two distinct traits; therefore, a high genetic correlation suggests that the two traits are influenced by the same genes in a comparable way and that genotypes would be constant in all environments. In the case of the marine alga Hormosira banksii, for instance, Clark et al. (2013) discovered positive genetic correlations between 120-hour-old embryos grown in control (20°C) and elevated (28°C) temperature treatments. This suggests that genotypes that did well in the control also performed well at elevated temperatures. Furthermore, for the sea urchin C. rodgersii, positive genetic correlations showed that the best-performing embryos in high-temperature settings also fared well in low-pH environments (Foo et al., 2012).

### 3.4. Genetic Correlation

The relationship between how one trait differs from another is known as genetic correlation. The attributes are positively associated if the correlated trait rises with the original trait. The qualities are negatively associated if the other trait declines as the first trait increases. The relationship between additive genetic effects is known as additive genetic correlation. This corresponds to the relationship between breeding values. When qualities are genetically correlated, it also means that their breeding values are associated. In this scenario, it is simple to determine the value of one feature based on the other. When a trait is chosen, the genetically connected trait will alter accordingly. When there is a positive connection, the selection of one feature will automatically lead to the selection of the other, accelerating the development of both qualities. Conversely, it is challenging to enhance both traits when there is a negative genetic correlation (such as that between milk production and milk protein percentage); index selection can help, but the process will take longer.

## 4. Conclusion

Growth hormone receptors (GHRs) have been found on the cell surfaces of many tissues throughout the body, including liver, muscle, adipose, and kidney, as well as in early embryonic and fetal tissue. Alignment of sequence data of 30 rabbits (Ten NZW, Ten Dutch, and Ten Hylamax). In addition, variations in growth rate or weight gain of rabbit within the same strain or among different strains could be attributed to environmental factors like nutrition, disease, hormone, and general management. Growth hormone receptors (GHR) are a transmembrane protein composed of 620 amino acids and are a member of the Type I cytokine receptor family of receptors.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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