



## Determination of New Short Sequence Repeat in IGF-1 Gene Associated with Maturity Body Weight in Selected Japanese Quail for Three Generations

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### Abstract

**T**HIS STUDY investigated the effects of selective breeding on maturity body weight (at 9-weeks of age) in Japanese quail across three generations. Descriptive statistics demonstrated a consistent upward trend in maturity body weight, with the mean increasing from 311.08 g in the first generation to 344.41 g in the third. Heritability estimated for growth traits suggested moderate potential for genetic improvement and the selection response of 18.38 g from Generation 1 to Generation 2 and 14.95 g from Generation 2 to Generation 3 indicated that the average body weight of the selected individuals increased by these amounts. The results showed that, from Generation 1 to Generation 2, the selection differential of 18.38 g reflected that the selected quail were on average 18.38 g heavier than the population mean from Generation 1. As the mean of maturity body weight increased from 311.08 g in the first generation to 344.41 g in the third, the corresponding estimated breeding values (EBVs) also reflected genetic gains, with values of 18.38 g and 14.95 g for generations two and three, respectively. The findings also identified a specific tandem repeat sequence, "CTCTCTCTCT," within the insulin-like growth factor-1 (IGF-1) gene, potentially serving as a genetic marker for predicting maturity body weight. The findings underscore the complexities of breeding strategies, as increased variability may complicate population management despite gains in mean maturity body weight. Overall the findings underscore the importance of balancing selection intensity and genetic diversity to sustain long-term genetic progress while optimizing maturity body weight traits in Japanese quail.

**Keywords:** Japanese quail, short-term selection, selection parameters, IGF-1 gene, SSR.

### Introduction

Japanese quail has emerged as another rapidly growing source of quality meat, especially in areas with an increasing need for poultry of smaller sizes. These birds also relished their meat as its composition is favorable, rich in proteins and minerals yet low in fats. The fact that it has a relatively short growth cycle usually attaining the market weight in between 5 to 6 weeks old makes Japanese quail an ideal species for commercial meat production [1-3]. They are comparatively smaller and hence can be intensively raised in areas where there is space constraint. Furthermore, Japanese quail rearing is also profitable to resource poor farmers and enhances food security, particularly in the under developed world and the Japanese quail meat is stable for meat production because they adapt meat

to various climate [2,4,5]. Because of their interactive size, they are less stressed due to overcrowding compared to bigger birds, thus decreasing mortality and increasing output. Further, the breeding cycle is quite short thus it is possible to rear a number of generations of quails in one year resulting to faster genetic improvement than the rest of the poultry [6].

Apart from being a source of meat, Japanese quails have also been shown to be a perfect animal model for investigating poultry selection. The rapid reproduction and quick generation time from 6 to 8 weeks from birth to sexual maturity, helps breeders and scientist see genetic selection effect after a few generations. It, simplifies the implementation and evaluation of selection criteria such as weight, feed conversion efficiency and age at first reproduction [7,8]. In poultry breeding, age at sexual maturity is

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an important phenotype characteristic that impact on reproduction as meat yield. This phenomenon may be preferable when selecting for the optimal age of maturity which would result in improved on growth rates, efficiencies of feed utilization and advancement in body weights at the market age.

For Japanese quail, the age at maturity is essential as it has been reported to influence body weight and the onset of egg production which is important in meat and egg dual purpose breeding programs [9]. Selecting birds with early maturity may shorten the duration to the market hence also lowering the cost of production and enhancing the number of generations that can be reproduced within a particular time frame. However, selection for earlier sexual maturity has to be done with a concern in mind, which is, ensuring that the birds in question attain an adequate body weight optimum for meat production. If sexual maturation is quicker, birds may have lower weights for production at maturity, which may demote their importance as meat producers to the farmer [10,11]. The Japanese quail has also been used for several years in experimental studies for evaluation of the genetic and physiological mechanisms control the growth of its various traits.

They resemble other poultry species in their genetic composition, which makes them an ideal research model aimed at enhancing the meat production qualities in wider avian taxa [8,12]. For Japanese quail, the focus on body weight at the age of maturity has been a dominant selection criterion for developed strategies of improving meat yield [13,14]. At the stage of sexual maturity Quail are usually selected on the basis of increase in body weight since this gives an assurance that in terms of reproduction and meat production, the birds are productive. During many generations of selection, breeders will be able to maximize utility of the entire generation by selecting a bird where reproductive capacity could be compromised but its weight is significantly higher [15].

For Japanese quail, the age of maturity is very important for the life span and general health as well. Those birds that sit a little earlier tend to be problematic later, because they vitiate their structural body with so much feeding, metabolic stress, and those who are a bit late tend to be less useful in terms of babies in the duration of the breeding life [16]. Likewise, the age of maturity is an important factor for the economics of quail farming. Birds that are brought into the market for sale after less feed consumption can thus be produced efficiently and this can result in the improvement of meat production systems further. Estimated targets' compliance is essential especially for the fast-growing birds lest they end being unprofitable costly birds [17-19].

In improvement programs through selective breeding, a heritable trait to research on is age at maturity since this will enable appropriate selection of individuals with the right genotype for the understanding of the increasing trait. The DNA-based tools such as SNPs and STRS have become important in the development of genetic markers relating to the age of maturity as well as bodyweight in quails [20,21]. There has been a shift in focus in the selective breeding programs with the incorporation of biotechnology DNA marker technologies particularly SNPs and STRS which have uncovered specific traits that marker Assisted Selection has been able to transform. Each SNP allele falls on a single base of DNA sequence, whereas an STR contains a single core sequence curious as many as 10 and more times. These markers have been widely used for the identification of genes that are height, the body weight and other economically significant traits in poultry [22].

In Japanese quail species, SNPs which were related to body weight and interfering with growth were present in the important candidate genes of growth regulating pathways. With the help of these markers, breeders are able to select birds that will reach a higher body weight at maturity making genetic advancement occur more rapidly than the conventional selection method which is based on selecting birds with favorable phenotypic traits. This strategy referred to as marker-assisted selection (MAS) accomplishes the objectives of breeding more efficiently and puts genetics in a positive trait discrimination [23,24]. One such gene that is known to regulate growth and body weight in birds is the insulin like growth factor 1 (IGF-1) hormone which involved in the regulation of growth and metabolism. In birds, IGF-1 controls body weight and muscle growth, and feed utilization efficiency, hence an important factor to be selected in meat production programs [23,25]. In case of Japanese quail, IGF-1 has been examined in relation to body weight gain and fat mass. For instance, birds with higher amounts of IGF-1 expression are known to grow faster and have higher weights when mature hence this gene becomes a target of selection in meat-production breeding programs.

Genetic variations within the IGF-1 gene, hypothetically SNPs, were correlated with nutrition-related traits making it possible for the aviary farmers to target the genetic potentials for better growth in the birds using the molecular markers [26]. In this case and with the introduction of IGF-1 as a genetic marker, the breeders will achieve better gains in body weight and growth rate for the Japanese quails, hence better meat production. This approach not only speeds up selection but also helps in achieving selection with higher understanding of the biological significance of the selection towards growth regulation [27,28]. This study aimed to explore selective breeding effects on maturity body

weight of Japanese quail screened for three generations using selection parameter. In addition to figure out the association between simple sequence repeat in IGF-1 gene and maturity body weight (at 9-weeks of age) in Japanese quail as potential genetic marker for predicting maturity body weight.

### **Material and Methods**

The study protocol was approved by Medical Research Ethics Committee (MREC) of National Research Centre, Egypt, under the ethical approval code (13050409). The relevant Egyptian laws and the Egyptian Drug Authority (EDA) and Ministry of Health and Population (MOHP) and Institutional Animal Care and Use Committee (IACUC) decrees, guidelines and recommendations shall be adopted and followed during the conduct of this research.

#### *Birds and managements*

The Japanese quail population was taken from the farm of the Animal Production Research Institute, Ministry of Agriculture, Egypt, and contains up to 600 birds. No traits will be selected in the population before the experiment begins. To make a selection (S) line and a control (C) line, birds were randomly chosen from the population and then allocated equally into two lines for reproduction which were individually leg-tagged. In one cage there were four females and one male meant for mating. Food and water were given ad libitum. Daily eggs were collected and labeled with a dam number to maintain pedigree records. After hatched, they were raised in group housing at 60 birds/m<sup>2</sup> floor space. Quails should have access to artificially lighted houses for 24 hours daily while being fed on a standard commercial feed containing 28% CP and 2,900 kcal ME/kg feed throughout the experimental period. Body weights at six weeks of age shall be analysed; superior birds will then be selected as parents for subsequent generations through random mating using a sire: dam ratio of one to four (1:4) which aims produce three generations (200 quail bird/each generation) [29].

Body weights at 9-weeks of age were analysed by an animal model to predict breeding values. The animal model allowed us predict breeding values by analysing body weight at 9-weeks. A constant ratio is used in generation replacement, and so selection intensity is a function of number of birds at each stage [30,31].

#### *Selection parameters*

The selection differential, selection response, selection intensity, realized heritability and estimated breeding value were calculated as [32] noted as follows; Selection Differential: The difference between the mean body weight of selected individuals and the base population.  $SD = X_p - X_0$  Where,  $X_p$  : the selected parents' mean, and  $X_0$  : the population mean. Selection Response: The change in

mean body weight from one generation to the next, attributable to selection. Selection Intensity: The degree to which the best individuals are selected compared to the average of the population.  $i = SD / \delta p$  Where, SD : the selection differential, and  $\delta p$ : the phenotypic standard deviation of the trait. Realized Heritability: The ratio of the selection response to the selection differential, indicating the proportion of the trait's variance that is due to genetic factors.  $h^2 = R / SD$  where, R: actual response of selection, and SD: the selection differential of previous generation. The breeding value (BV) is an estimate of an individual's genetic gain, particularly for a specific trait, such as body weight at a certain age. In a selection program, breeding values are often calculated using the formula:  $EBV = \text{Heritability} * (\text{Observed Trait Value} - \text{Mean Trait Value})$  and  $EBV = h^2 * (\text{Selection Differential})$ .

#### *Genomic DNA Extraction*

Genomic DNA was extracted from blood with the use of an appropriate DNA blood Kit (Bioneer Inc., Daejeon, Republic of Korea) according to the manufacturer protocol. The DNA quality was estimated by a spectrophotometer with purity ratio of (1.8).

#### *PCR amplification*

The PCR reaction performed using two primers to amplify the insulin-like growth factor-1 (IGF-1) hormone using primers as follows: F: 5'-TTTGCCAGAAGAGGGAGAGA-3'; R: 5'-GCAGAAGCAGACAACACACA-3, with size product 418 bp [26]. PCR amplification reactions were carried out in 50  $\mu$ l of overall volume that comprised 50 ng of template DNA, 10 pmol of every primer, 0.25 U of Taq DNA polymerase, 10 mM of Tris- HCl (pH.9.0), 250 mM of dNTPs mix, 30 mM of KCl, 1.5 mM of MgCl<sub>2</sub> and sterile nuclease free water to obtain the ultimate volume of 50 ml. The following cycling conditions were used to carry out the PCR reaction: pre-denaturing at 94°C for 5 minutes, denaturing at 94°C for 30 seconds, annealing at an appropriate (56 °C) for 30 seconds and extension at 72°C for 30 seconds for 35 cycles and finally, an extension at 72°C for 10 minutes. An analysis of the amplified fragments was carried out in 1.5% agarose stained with ethidium bromide. In addition, a 100 bp DNA ladder was added in agarose gel electrophoresis to examine the size of amplicon product. Gel documentation system was used to obtain the images, and the software available with the gel documentation system was used to determine the size of the amplicon.

#### *Statistical Analysis*

Bioneer Inc. (Daejeon, Republic of Korea) purified PCR products and sequenced them with 40 samples for each generation. CLUSTALW 2.0.12 was used to perform multiple sequence alignments so as to detect conserved regions and possible

polymorphic sites among collected sequences. The sequences obtained were then subjected to similarity searches using BLAST programs from NCBI against Genbank database. SAS statistical package (Version 11, 2013), provided robust framework for analysing the data statistically. The model applied is as follows:

$$Y_{ij} = \mu + B_i + e_{ij}$$

$Y_{ij}$  is the observation taken on body weight at maturity (9 weeks),  $\mu$  is the overall mean,  $B_i$  is vector of the fixed effect of the breed,  $e_{ij}$  is vector of the random error assumed to be independent normally distributed with mean = 0 and variance =  $\sigma^2$ .

## Results

### *Descriptive statistics of maturity body weight*

The descriptive statistics for the maturity body weight trait across three generations of Japanese quail have a clear predicted evolution of body weight. This was also depicted by the mean, median and mode while variability measures such as standard deviation, range, help in enhancing the understanding of the distribution and dispersion of body weight through generations. Whereas concerns with mean body weight, the mean body weight was the lowest (311.08 g), which indicated that the first or initial population of quail had the lowest average body weight, there was however a noticeable increase in mean body weight (329.46 g) with indications of selecting or breeding in practices that are positively increasing weight.

Additionally, it was observed that the mean body weight continued to improve showed a positive direction of body weight changes across generations until the third generation reached 344.41 g up weighted for selectively bred quails. This indicated that the improvement was not only sustained but also accelerating, suggesting effective breeding practices and selection methods. There has been a consistent mean which keep on improving over generations which suggested that there was effective selection or breeding practices on body weight enhancement. This increase indicated progress in breeding objectives focusing on improvement of size and weight.

The standard deviation, which, the smallest (14.35 g), showed lesser degree of variation ranges in body weight of quail birds belonging the first generation. The value of the standard deviation increased further (24.25 g), implying that there were more body weight variation as ocular average body weight increases. However, the most advance standard deviation (31.60 g) also exhibited the most body weight variability and this showed that some selection or breeding methods created a wider depth in body weights. The widening of the standard deviation on the other hand indicated that while the mean body weight was improving, more variation was being experienced among different bodies. This

could be due to the higher selection for body weights resulting in a wider body weight range.

The increasing standard error over the successive generations (from (2.27 g) to (5.77 g)) could be due to the increasing variability and the range of body weights shown on table 1. The reinforcing direction of the mean body weight across generations was supported by the rising median value which is as a result of crossing over with the other population. The place of the median in relation to the mean further supported the data and showed that body weight was becoming more highly positively skewed as central values get higher.

Similarly, the transition from plural modes in Generation 1 to simple mode in Generation 3 suggested that the distribution of body wise has shifted towards a higher mean. Similarly, the results obtained from our study noted that the growing span across generations indicated that on average body weight is increasing, but also there is increasing heterogeneity in body weight of the quail. This might be an outcome of the broadening of the genetic stock or intensification of selection for different traits. The data depicted an effective breeding program to enhance maturity weight of the Japanese quail over three successive generations. There was thus a consistent rise in the mean body weight as a clear indicator of successful selection pressure.

### *Genetic parameters for maturity body in Japanese quail*

For selection differential parameter, the difference in mean body weight between the selected individual and that of the above generation's mean body weight. It decreased from Generation 1 to Generation 2 to Generation 3, which could indicate either decline in the selection pressure or relocation of the population. On the issue of selection response, the results reflected how there was an increase in the average mean body weight from one generation to another. It reflects the efficiency of the selection process. In both transitions the response is high, meaning the selection has been effective.

Though, such reductions in selection intensity were demonstrated by Generation 1 to Generation 2 to Generation 3. Less intense selection in subsequent generations meant that the population either was selected more evenly or the pool of individuals out of whom selected individuals were fewer. Besides, where heritability was measured despite being high could reflect appropriate selection techniques or high negative genetic correlation of body weight with selected traits indicating that this trait has been maximally expressed during the selection of individuals. Lastly, it was evident from the data that body weight selection in Japanese quails has worked effectively for many generations.

Currently, although the selection response remains at a high level, selection intensity decreased,

and this may reflect changes in the selection as well as demographic characteristics of the population. The increase of the variance of the selected breeding values (BV) occurred when there is high realized heritability. In this case, high realized heritability (a measured value close to 1.00) suggested that all the selection response achieved over a given period was genetic rather than the environment-induced. To illustrate, if the heritability is very high, the BV computed will in reality depict the gain that can be expected in the next generation that has been derived from the development undertaken. The values of realized heritability indicates that majority of the treated body weight changes were as a result of changes at the genotype level.

As shown in Table 3, the Estimated Breeding Value (BV) attempts to quantify the ability of a certain trait, for instance, body weight in adulthood from the mean of the population. It indicated that the relative worth of an animal for use in breeding to the potential breeding values that could be achieved in an average animal. The estimated breeding value was 18.38 g, indicated that generation 2 selected individuals had an average body weight that was 18.38 g more than the average body weight of generation 1. The same trend was observed in generation 3, the estimated breeding value is 14.95 g, which means generation 3 selected individual had an average body weight of 14.95 g more than the average body weight of generation 2. These figures represent an improvement in genetics over generations due to selection methods. High values of BV denote great increase in body weight from the selection processes carried out.

#### *Relationship between breeding value and selection parameters*

To summarize evaluation of genetic progress and selection procedures in the breeding program for Japanese quail, table 4 provided a clear summary of the metrics. In the long run, a larger selection differential usually predicts a BV as it meant that the selected individuals were quite productive than the average population. From Generation 1 to Generation 2, the selection differential of 18.38 g captured that the selected quails were on average 18.38 g heavier than the population average in Generation 1. This was directly reflected in the BV calculation of Generation 2. High EBVs are an indicator of effective breeding programs and have been associated with impressive improvement in growth characteristics in birds over other studies.

The selection response indicated how much the selection has been effective. A generation of higher selection response oftentimes produces a higher BV in the next generation. The selection response of 18.38 g from Generation 1 to Generation 2 and 14.95 g from Generation 2 to Generation 3 conversely indicated the amount of average body weight increase of the selected individuals. This increment

was particularly apparent in the BV taken out, showed that the breeding value of individuals has considerably enhanced. As regards to the realized heritability from Generation 1 to generation 2 was (0.91) some will argue that the majority of the response to selection is genetic with little environmental effect.

Similar outcomes were observed also throughout generations two and three, particularly (0.83) which is relatively high but lower than that in the previous generation, indicating further environmental impacts or saturation in genetic improvement. Strongest selection intensity was most times associated with increase BVs as these were the individualised who were selected out of much better individuals than average. The increase in selection intensity led to the greater differential between the selected individuals and the average population increasing the estimated breeding values. Considerable selection intensity in the early generations as well as adequate realized heritability was taken to mean effective selection and improvement of the young population. Declined selection pressure as well as minor decline of heritability values in subsequent generations pointed out the corrective measures to be formulated due to limiting expected genetic gains.

#### *Sequence tandem repeat in IGF-1 gene*

Also, the specific tandem repeat that exists in all of the individuals can have a major influence on the other phenotypic characteristics targeted in this study especially the body weight of Japanese quail. This requires more functional analyses to see how changes or variations of this repeat will affect the expression of the IGF-1 and the growth performance in poultry selection. Verification of the effect of this tandem repeat polymorphism on IGF1 expression will enable us devise ways of using genetic selection to enhance the quail's growth traits. Our results noted that a particular simple sequence repeat was identified as a tandem repeat sequence in the segment of insulin-like growth factor-1 gene in selected lines of Japanese quail for maturity body weight.

The findings found that the repeat unit "CT" occurs in all the sequences examined and the majority of the associated tandem repeat consists of "CT" with longer stretches of CT (10bp) repeats. The repeat sequence is common to all the sequences as it was reported that the repeat unit "CTCTCTCTCT" starts at position 359 across all sequences and that the ending position of this repeat unit was consistently at position 368 in all sequences. Each sequence possesses only one copy of the "CTCTCTCTCT" tandem repeat.

#### **Discussion**

In the recent past, the Japanese quail (*Coturnix japonica*) has emerged as one of the most sought – after species of livestock for meat and as a model organism in specific breeding programs. The

Japanese quail, with its small size, short generation interval, high reproductive rate, and fast growth rate, is perfectly adapted to the research on genetic enhancement and in commercial meat production [33,34]. In particular, unlike large poultry species, like chickens and turkeys, quails and their breeding programs require lesser management, relatively smaller area, and due to their shorter reproductive cycle, faster generation turnover renders them easier for selective breeding programs [2]. Another important aspect is that quail meat is healthy and nutritious and there is an increase in demand for it which further warrants the development of genetically altered quail strains that cater for meat production attributes like body weight and maturity age [4,35].

The selective breeding has always been a mainstay of animal agriculture, assisting in the improvement of the productive parameters such as the growth rate, feed conversion efficiency and the quality of the carcasses. Selection at maturity age has increasingly become of importance for breeding programs targeting the increase of productivity. Given their well-researched lifecycle, that of the Japanese quail has been instrumental in the examination of age at sexual maturity and body weight and how that can translate to increased genetic selection for meat production in poultry [36,37].

#### *Statistical analysis of maturity body weight*

The analysis of body weight in Japanese quail over a period of three generations provided insightful information on how selective breeding has affected growth characteristics. Age trends as regards the adult body weight of birds revealed that there has been a steady increase in mean, median and mode values over the years. Although the first-generation average weight of 311.08 g represented a starting point, average weight progressed significantly over generations culminating in an average body weight of 344.41 g in the third generation. This type of development not only showed improvements concerning the breeding appertaining to the targets of size and weight enhancement but rather mechanized selection procedures that have worked over the generations [38-40].

In addition, variance analysis, standard deviation, range, dispersion index among other measures also help to characterize the body weight patterns within a population. For the first generation, standard deviation was lower, having a value of 14.35 g, a factor which indicates little difference in individuals. With the increase in mean values of average body weight, the standard deviation was also elevated to 31.60 g in the 3rd generation. This direction meant wider range of body weights observed suggested enhanced selection pressure led to more heterogenous population. So, the breeding effort has produced a diversified population with animals

exhibiting a greater variety of body weights. This variety may be advantageous for adapting to diverse environmental situations or for particular breeding aims, since it provides for more genetic variation from which to pick future breeding prospects. Increasing selection pressure most likely pushed the limits of tolerable body weights, resulting in increasing variability [38].

Similar trend of enhancement of parameters of traits selected has been observed in other investigation work done on quails and other birds' Similar trends of increased variability of performance traits also seemed to increase probably in this case selection is being applied for many traits as well because performance is very often predetermined by multiple genetic varieties [38], so increasing genetic variety of left to right profiles as a counter action to selection. Progressed from bimodal distribution in first generation to unimodal in third generation further goes to show how solid body weights progressively becomes exposes towards the higher values and contributed positively in the gains achieved through the breeding program.

But over these many years of observed improvements in mean body weight and the corresponding breeding strategies the concern however has been the higher variability and heritability factors that comes with such a diverse population management [41,42]. This study showed a need to pursue the larger body weight targets and do it in such a way as to understand the degree of variation in the performance that accompanied such strategies due to intense selection in weight increase and other features of selected.

As a whole the results added to the knowledge about the weight dynamics in Japanese quails as well as about the issue of selective breeding and its consequences in terms of genetic variability and population sustainability [13,43,44]. Body weight increments that were observed should also be associated with these genetic changes to seek the underlying causes of such changes. The heritability of growth traits demonstrated moderate success for genetic gain which meant that more selections could offer opportunities in better traits including egg production and overall health of the subject [45]. In addition to this, it is also supported by other studies that increasing weight could be associated to increase the traits' productivity however, there are consequences as well, survival and reproduction will always be the competing factors [46-48].

When the first-generation values of the standard deviation 14.35 g, it meant that there was less variation in body weight among quails, and as generation increases there is an increase in standard deviation 24.25 g, where more body weight was expected as the mean average weight increases. On the other hand, the highest standard deviation 31.60 g signified that there was also a greater range of body

weight among individual quails than uniform selections or breeding practices have been used. The growing trend of standard deviation showed that while the average indicated improvement in body weights, this was probably as a result of higher selection pressure which may create wider body weight diversity within the population. As the generation progressed, the generation mean body weight increased (from 2.27g to 5.77g), there was noticeable increase in generation standard error (2.27g to 5.77g). This could be the explanation of the increase in generation standard error over generations. While, with regards to median values, the escalated median value with rising generations supports the increase in body weight trend [49].

The body weight distribution has become increasingly positively skewed in the central values, as shown by the position of the mean relative to the median. The transition from multiplicity of modes in generation 1 to a unimodal mode in generation 3 indicated that the distribution of body weight has become much narrowed around the larger value. Further, our findings also showed that the widening range of generations over the generations implied that even though average body weight of quail is increasing, there is also more variation in body weight among the quail. The results depicted an effective breeding program for enhancing maturity body weight in Japanese quails for three generations. There was a steady increase in the mean body weight which indicates efficient selection in the population. However, there were increased dispersion across the body weight measurements as shown by the higher standard deviation and range. Variance due to this variability increases making it difficult to achieve accuracy as indicated by the increasing standard error. The changing from the several modes to one mode and from one range to another range illustrate dynamics in the distribution of body weight [50,51].

The widening span of body weights over the generations is also in favor of the evidence that, whereas the average body weight is increasing, the average genetic pool has likely broadened for example because of the inclusion of various selection criteria or traits that are not directly associated with body weight. Such increased diversity needs to be managed to prevent adverse consequences such as undesirable body weight extremes or loss of genetic vigor in other essential characteristics [32]. As the issues of selective breeding for Japanese quail become more and more complicated, it is necessary to consider not only external characteristics but also genetic relationships underlying the progress of these traits. In recent investigations, it has been demonstrated a significant correlation of egg weight with negative consequences of high mortality, indicating that the enhancement of body weight might lead to negative reproductive success in subsequent generations [48].

This emphasized the importance of incorporating genetic diversity in targeted selection approaches for the breeders which are limited in that desirable traits would be increased but overall population fitness would still be maintained. Moreover, several estimates of heritability show stage-specific differences, implying that any attempts to maximize early growth will be beneficial with respect to survivorship and economic returns on phenotype traits [45]. This situation reinforces the continuous research on the genetic makeup of quail populations to foster informed breeding programs that address short term profitability and sustainable long-term goals [44,45,48,52].

#### *Genetic parameters for maturity body in Japanese quail*

The selection differential, which refers to the selective breeding for specific traits ending in a certain body weight for the improve generation(s) relative to the mean body weight of the proceeding, started off high in Generation 1 but dropped with Generation 3. This drop could mean less force applied to the selection or more of a change in the genomic landscape of the population. As ranges of heritability remained constant within a population over generations, they usually tended to follow a reduction, due to increased selection, hence the average body weight is fast approaching the ceiling, or perhaps there is a concentrated loss of diversity within the population such mired in selection and progress towards final selection is so forgone [32].

Selection intensity also decreased across generations, implying that extreme individuals were tending to be selected only less or more of a selection pressure was applied as the population may have been genetically homogenized by previous selection efforts. When there is genetic variance, there is high selection pressure. However, as selection pressure increases, such individuals will be harder to find as there are less individuals with high genetic potential for that particular trait converting it into a decreased selection intensity [53].

Among the most significant observations was the high heritability estimate for body weight, which implies that a great deal of the variation in body weight is genetically determined. This high heritability here indicates that the selection approach has worked to harness the genetic capability for body weight enhancement. Heritability estimated close to 1.00, like the ones in this study, show that a huge portion of the variation of the trait can be traced to the genetic makeup, and the modulation of body weight by other factors is insignificant. Other high heritability values have been recorded in such studies with quail and poultry suggested that weight gains are improved on genetically [54].

The Estimated Breeding Values (EBV) of maturity body weight characterized genetic improvement based on relative average population

genetic potential of individuals. In Generation 2 the EBV value obtained was 18.38 g which inferred that the selected individuals were expected to weight 18.38 g more than what was the population of Generation 1. In Generation 3, the EBV was again 14.95 g, further suggesting genetic improvement. High EBVs are an indicator of effective breeding programs and have been associated with impressive improvement in growth characteristics in birds over other studies [55]. Generally, the findings indicated that the maturity body weight selection program for the Japanese quail has been fairly successful with respect to genetic improvement being made over that time period. Still, the declining selection differential and selection intensity reflect the inevitable difficulties of selection schemes implemented over a long time period such as loss of genetic diversity and population homogenization.

#### *Relationship Between Breeding Value and Selection Parameters*

Finding breeding value (BV) and the selection parameters with respect to body weight in Japanese quail indicated great relevance of the accuracy of the breeding program. It is known that the greater the selection differential which is the measure of the deviation of the selected individuals from the population average assuming that they are above average, the greater the BVs. Of this direction, the transition observed between Generation 1 to Generation 2 illustrated more. Where a selection differential of 18.38 g where in turn triggered an improvement in the breeding value. The higher selection differential also implied that the individuals that were selected were on average more, rather than less than average and therefore contributed to faster rates of genetic improvement than the population did. This phenomenon has been reported in other poultry studies arguing that higher selection differentials have hastened the achievement of BVs and genetic improvement [56].

In our results, the selection responses of 18.38 g from Generation 1 to 2 and 14.95 g from Generation 2 to 3 are reflected directly into the BVs. These results confer that the applied breeding strategy has worked in the sense of facilitating selection and turning it into genetic gain. The positive selection response in both transitions observed in these generations further confirms and highlights the efficiency of the selection program with similar outcomes reporting that consistent selection over time can lead to enhanced genetic body weight improvement [57]. The realization heritability in response to selection which was attributed to genetics was really high in all the generations with 0.91 and 0.83 from Generation1 to Generation2 and Generation2 to Generation3 respectively. The high heritability suggested that body weight enhancing traits were respondents to most of the interventions genetically with little to no effect from the external

factors. There was of course the fatigue on heritability from Generation 2 to 3. However, the level of heritability remains high, which means that genetic management of breeding continues to have the upper hand in selection response. The decreased heritability can somehow be interpreted as higher environmental effects or that the population is nearing the ceiling of its genetic response, which has generally been the case in long term selection programs [58].

The higher selection intensity, that measures how much improvement the chosen individuals are when compared to the population, was persistent in the early generations improving the BVs. Because of improvement in selection intensity in the later generations, the BVs also recorded a slight improvement. The cause of this decline in selection intensity could be the advancement of the selection process that led to a more homogenous population or limitation of genetic variability. A careful and appropriate handling of selection intensity is significant in sustaining the rate of genetic improvement since a drastic fall may hinder progress in addition gains the future [53]. The conclusions drawn from the maturity body weight trait breeding program are especially positive, although it may be proposed to change the selection efforts or includes new genetic backgrounds so as to maintain progress in successive generations. Quite similar conclusions have been drawn in other investigations which documented the importance of progressive change in breeding designs so as to improve genetic progress with no gains in plateau [59].

#### *Sequence tandem repeat in IGF-1 gene*

Molecular markers have been in use for long in determining the extent and utilization of genetic diversity in plants and animals. Focusing on MAS putting forward recurrent selection and genome-wide association analysis (MARS and GWA respectively) are seen as future strategies for polishing complex traits in plants and animals [60]. The emergence of or introduction molecular genetic research has become more fashionable and for this comprises of projecting many more molecular genetic markers that would find polymorphisms of traits which have an economic value [61].

Additionally, due to the development in molecular genetics and DNA technologies such as SNPs and STRs, selective breeding procedures have changed beyond recognition. The tools enable the breeders to pinpoint the genetic markers that govern economically relevant features such as maturity body weight and growth rate [27]. One of the significant genes responsible for growth and meat production in birds is the IGF-1 gene [23]. IGF-1 is part of the system for growth and metabolism adjustment, therefore it also provides a good endpoint during attempts to enhance meat production in Japanese quails and other poultry programs [27].



The tandem repeat sequences located within the insulin-like growth factor-1 (IGF-1) gene can be a factor that influences the phenotypic characteristics of Japanese quail, for example, body weight. There were tandem repeats which can be found within the CTCTCTCTCT sequence important as illustrated in the study. These were also short repeating sequences regarded to be involved in many biological functions such as growth, and development, through change in the expression of genes or their proteins [62,63]. Given findings revealed a constant "CTCTCTCTCT" tandem repeat unit of IGF-1 gene for all sampled sequences of maturity body weight selected quail.

This repeat encompassed the region from positions 359 to 368, which include the stretch of 10 bp extending "CT" short sequences of the nucleotide. The fact that this repeat has been found in all the sequences indicated that it may be responsible for the differences in growth traits particularly body weight. It seemed that the tandem repeat sequence of 'CTCTCTCTCT' may be the candidate for maturity body weight attitude marker for Japanese quail because it possesses the features of the variable number tandem repeats VNTRs. VNTRs are a class of genetic markers containing repeating sequences in head to tail arrangement, which may be related to various phenotypic traits such as body weight [64].

The repeated sequence of 'CTCTCTCTCT' may be more widely understood as a genomic marker for body weight predictions however, the existing literature focuses more on SNPs and body measurements. These studies contribute to the development of complex understanding of the genetics underlying these traits [65,66]. They revealed the presence of certain candidate genes related to body weight and their possible role as markers for selection aiming to enhance growth performance [66]. Some studies have quantified body weight positively correlating with body girth and thigh length, linear dimensional measurements, and therefore would predict body mass despite non-performance being exercised [67, 68].

The use of multiple regression analysis emphasized the major role of body weight in the linear dimensions of the body evidenced by body weights which indicated that these indicators can be alternative methods of predicting weight [67-70]. While the repeat sequence 'CTCTCTCTCT' has not been directly addressed before, the findings suggested that genetic markers, including SNPs and body measurements, are critical for predicting body weight in Japanese quail. Further research is needed to explore the specific role of tandem repeats in IGF-1 in quails. It is essential to carry out further work to understand the tandem repeats' involvement referencing IGF-1 in quails. Furthermore, such comprehensive analysis of these genetic polymorphisms and environmental interactions will enhance our understanding of the phenotypic outcomes brought about by these factors.

Differences among growth traits if described based on genetic variation would not be conclusive due to the vast role played by other variables, environmental factors for instance nutrition and management. It was found that the effects brought about by environment on the expression of growth-related genes such as IGF-1, which indicated that current breeding strategies deal with these issues quite well [68]. Overall, the present study results widen the scope of future investigations of the genetics of growth control in Japanese quail. Understanding the association of traits like maturity body weight and feed efficiency with MAS such as tandem repeat variations in IGF-1 could also be beneficial for enhancing genetic selection programs. The information on genetic markers that enhance desirable growth traits would assist breeders in improving selection efficiency in poultry production through promoting better and fastest growth.

### Conclusion

The present study assessed the influence of selective breeding on the maturity weight in Japanese quail over three generations thus turning growth traits and genetic diversity as major focus areas. Linear progression for maturity body weight continued steadily improved. It was shown that the first generation, maturity body weight continued to increase from 311.08 g in the first generation to 344.41 g in the third generation. From Generation 1 to 2, the selection response was 18.38 g. The selection response dropped in Generation 2 to 3 when 14.95 g was obtained which reflects useful genetic improvement. Moderate estimated heritability was found for maturity body weight. Additionally, a specific tandem repeat sequence, "CTCTCTCTCT," within the IGF-1 gene was identified as a potential genetic marker for predicting maturity body weight.

### Author Contributions

H.A.Y. and S.F. contributed to the study design, performed experiments, analysed the data, and wrote the manuscript; H.D and S.F. contributed to the data analysis and wrote the draft of manuscript; M.H. Abdelfattah and M. I. Badawy contributed to bird management; H.A.Y. contributed to DNA extraction and performed PCR, and finalized the manuscript; All authors have read and agreed to the published version of the manuscript.

### Conflict of interest

All the authors declare no conflict of interest.

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**TABLE 1. Descriptive analysis for maturity body weight across three Generations**

Statistic	Generation 1	Generation 2	Generation 3
Mean	311.08	329.46	344.41
Standard Deviation (SD)	14.35	24.25	31.60
Standard Error (SE)	2.27	3.83	5.77
Median	312.875	333.17	347.42
Mode	294.25, 304.5, 312.75	None	347.3333333
Range	64.5	106.75	144.75

**TABLE 2. The section differential, selection response, selection intensity and realized heritability in selected Japanese quail for maturity body weight.**

Item	Generation 1 to Generation 2	Generation 2 to Generation 3
Selection Differential (S)	18.38 g	14.95 g
Selection Response (R)	18.38 g	14.95 g
Selection Intensity (i)	1.28	0.62
Realized Heritability ( $h^2$ )	1.00	1.00

$h^2 = R/S$ ; R: response to selection; S: Selection differential

$R = h^2 \times S$ ; R: response to selection;  $h^2$ : realized heritability; S: Selection differential

$i = S / \sigma_{BW}$ ; S: Selection differential;  $\sigma_{BW}$ : standard deviation of body weight at 9 wks

**TABLE 3. Estimated Breeding Values**

Generation Transition	Estimated Breeding Value (BV)
Generation 1 to Generation 2	18.38 g
Generation 2 to Generation 3	14.95 g

$EBV = \text{Mean} + h^2 \times (\text{Selection Differential} - \text{Population Mean})$

**TABLE 4. Integration of Selection Intensity, Realized Heritability, and Estimated Breeding Value**

Generation	Selection Intensity (i)	Realized Heritability ( $h^2$ )	Estimated Breeding Value (BV)
Generation 1 to Generation 2	1.28	0.91	16.73 g
Generation 2 to Generation 3	0.62	0.83	12.43 g

$h^2 = R/S$ ; R: response to selection; S: Selection differential

$i = S / \sigma_{BW}$ ; S: Selection differential;  $\sigma_{BW}$ : standard deviation of body weight at 9 wks

$EBV = h^2 \times (\text{Selection Differential})$

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## تحديد تسلسل متكرر قصير جديد في جين (IGF-1) مرتبط بالوزن الناضج في السمان الياباني المنتخبة لثلاثة أجيال

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### الملخص

تهدف هذه الدراسة إلى دراسة تأثير التربية الانتخابية على الوزن الناضج في السمان الياباني عبر ثلاثة أجيال. أظهرت النتائج رؤية مهمة حول صفات النمو والتنوع الجيني. وأوضحت الإحصاءات الوصفية وجود اتجاه تصاعدي ثابت في الوزن الناضج، حيث ارتفع المتوسط من 311.08 جراماً في الجيل الأول إلى 344.41 جراماً في الجيل الثالث. أفادت النتائج أن العمق وراثي المقدر لصفات النمو ذو قدرة متوسطة لتحسين الصفات الوراثية، مما يبرز أهمية الموازنة بين الانتخاب لزيادة الوزن والحفاظ على التنوع الوراثي في هذه السلالات. أظهر استجابة الانتخاب زيادات مقدارها 18.38 جراماً من الجيل الأول إلى الجيل الثاني و14.95 جراماً من الجيل الثاني إلى الجيل الثالث، ما يعكس أن متوسط وزن الأفراد المنتخبة زاد بهذه القيم. أظهرت النتائج أن الفارق الانتخابي البالغ 18.38 جراماً من الجيل الأول إلى الثاني يعكس أن السمان المنتخبة كان أثقل بمقدار 18.38 جراماً عن متوسط وزن الجيل الأول. ومع زيادة متوسط الوزن الناضج بمقدار 311.08 جراماً في الجيل الأول إلى 344.41 جراماً في الجيل الثالث، عكست القيم التربوية المقدر (EBVs) أيضاً زيادات مرتبطة وراثية، بقيم 18.38 جراماً و14.95 جراماً للأجيال الثانية والثالثة على التوالي. أكدت النتائج المتحصل عليها من القيم التربوية المقدر ذات القياسات المرتفعة أن العوامل الوراثية تلعب دوراً مهماً في هذه التحسينات الملحوظة. كما تم تحديد تسلسل متكرر قصير (SSR) "CTCTCTCTCT" ضمن جين (IGF-1)، مما يشير إلى إمكانية استخدامه كعلامة وراثية للتمييز بالوزن الناضج بالسمان الياباني. بشكل عام، تبرز النتائج أهمية الموازنة بين شدة الانتخاب والتنوع الجيني للحفاظ على التحسين الوراثي طويل الأمد، مع تحسين صفات الوزن الناضج في السمان الياباني.

**الكلمات الدالة:** السمان الياباني، الانتخاب قصير الأمد، عناصر الانتخاب، جين IGF-1 ، SSR .