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GENETIC AND PHENOTYPIC TRENDS FOR BIRTH AND WEANING WEIGHTS AND THE MOLECULAR ASSOCIATIONS OF THESE WEIGHTS WITH *GH, PRL* AND *FSHR* GENES IN THE EGYPTIAN BUFFALO

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SUMMARY

The objectives of this study were to estimate heritability using single trait animal model and plot the genetic and phenotypic trends for birth weight (BW), weaning weight (WW) and daily weight gains (DG) of the Egyptian buffalo, and to detect the molecular associations of these traits with SNP genotypes of GH, PRL and FSHR candidate genes using PCR-RFLP technique. Data of growth traits were collected from 8229 buffalo calves, in El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S) herds belonged to the APRI, Ministry of Agriculture, Egypt. For the molecular associations analyses data were collected from 174 animals in three herds only. The regressions of predicted breeding values of BW, WW and DG on year of birth indicated slight increase in genetic trends over time (1.6 to 1.8 kg for BW, -0.519 to 1.57 kg for WW and -24 to 18 g for DG). The phenotypic trends showed slight decrease in BW, WW and DG over years of birth (36.6 to 32.9 kg for BW, 94.55 to 94.15 kg for WW and 628 to 582 g for DG). The GLM favored significantly TC relative to CC genotypes of GH gene. The GLM analyses for BW, WW and DG favored significantly AA relative to GG genotype of PRL gene. The GLM differences among these genotypes for BW, WW and DG were significantly in favour of GG genotype relative to GC and CC genotypes for FSHR gene in NG and NK herds.

Keywords: Egyptian buffalo, body weights and gains, genetic and phenotypic trends, GH and PRL and FSHR genes, PCR-RFLP

INTRODUCTION

An ultimate goal in buffalo breeding is to rank the breeding animals according to their genetic merit for the relevant growth traits and use them efficiently in breeding programs. The genetic evaluation of buffalo calves is, therefore, a key issue to identify the superior genetic calves in a herd. Assessment of the predicted breeding values (PBVs) is an essential step for genetic improvement programs of the buffalo (Meyer, 2004). Accordingly, the package of BLUPF90 software (Misztal *et al*., 2018; http://nee.ads.uga.edu//wiku///doku.php) is known as a worldwide standard methodology for the prediction of the PBV for economic traits of farm animals. In evaluating the breeding programs in Egyptian buffalo, the genetic parameters for growth traits (*i.e*. heritability and PBV) are needed to be evaluated accurately to predict the genetic and phenotypic trends for the traits of concern and consequently to evaluate accurately the breeding programs of Egyptian buffalo. Most of the Egyptian research articles showed that the genetic and phenotypic trends of body weights were favorable positive and showing an increase in both trends for Egyptian buffalo (El-Bramony, 2014; El-Sayed *et al*., 2020 and Salem *et al*., 2021) and were like those of the Indian buffalo (Malhado *et al*., 2007 and Gupta *et al*., 2015).

Over the last decade, some molecular studies on buffaloes provided molecular markers to be used in marker assisted selection (MAS). This led to improve the selection response of growth traits in buffalo (Abo Al-Ela *et al*., 2014; Kathiravan *et al*., 2019; Erdoğan *et al.*, 2021 and El-Magd *et al.*, 2021). The molecular studies conducted on Egyptian buffalo showed that Growth Hormone gene (*GH*) can serve as a candidate gene for the genetic improvement of growth traits as it is known to have several biological functions such as water and electrolyte balance support, enhancing milk production and reproductive functions stimulation (Othman *et al*., 2012a and Darwish *et al.*, 2016). Moreover, studies on non-Egyptian buffalo revealed the polymorphic associations between the candidate *GH* gene and growth and carcass traits (Konca and Akyüz, 2017; Eriani *et al*., 2019; Nafiu *et al*., 2020 and Özkan *et al*. 2020). Similarly, Prolactin gene (*PRL*) was recognized to have multi-biological functions such as water and electrolyte balance, growth and development, immune and reproductive functions (Othman *et al*., 2012b; Konca and Akyüz, 2017 and El-Magd *et al.*, 2021). Furthermore, Follicle Stimulating Hormone Receptor gene (*FSHR*) was found to be an important candidate gene for development, growth, differentiation and maturation in buffalo (Chu *et al*., 2012). Thus, *GH*, *PRL* and *FSHR* genes proved to be growth encouraging factors and can serve as candidate genes to identify the molecular markers associated with growth traits for selection programs in buffaloes. Yet, studies on the variability and association among these candidate genes and growth traits still limited in Egyptian

buffalo. Therefore, the aims of the current study were to: 1) Estimate the variance components and heritability for some growth traits in the Egyptian buffalo using Bayesian Gibbs Sampling Algorithm, 2) Predict the breeding values and plot the genetic and phenotypic trends for body weights and gains using BLUPF90 software, and 3) Detect the molecular associations among *SNP* genotypes of *GH*, *PRL* and *FSHR* candidate genes and body weights and gains in Egyptian buffalo using PCR-RFLP technique.

MATERIALS AND METHODS

Buffalo herds studied:

Six experimental buffalo herds were used in this study. These herds belong to Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt, nominated as El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S). The herds NG and NK are located in Kafr El-Sheikh Governorate, while EN herd is located in Behira Governorate, EG herd is in Gharbia Governorate, ES herd is in Damietta Governorate and S herd is in Beni Suef Governorate. All herds are situated in Lower Egypt except Sids herd is located in Upper Egypt.

Management and feeding:

Buffaloes were kept under semi-open sheds; heifers were joined for the first service when reaching 24 months of age or 330 kg in body weight. Buffaloes were fed Egyptian Berseem (*Trifolium alexandrinum*) along with varying amounts of integrated concentrate feed mixture (48% decorticated cotton seed cake, 21 % wheat bran, 20 % maize, 5 % rice polish, 3 % molasses, 2 % limestone, and 1 % sodium chloride) according to the feeding regime of APRI. The diet contains 16 % protein for buffaloes and heifers and 17 % protein for suckling calves up to 6 months of age. Feed offered manually; roughage diets (silage - rice straw - alfalfa – alfalfa hay) are served first, followed by the concentrate diets. Feeding takes place twice a day at six AM and five PM. The calves were fed colostrum for the first three days after birth at 3% of their body weight, weighed individually within the same day of birth to record birth weight (BW, *kg*), and weighed to record weaning weight (WW, *kg*) after 105 days from birth. To obtain daily weight gain (DG, *kg*/d), BW was subtracted from WW and then divided by 105.

Data structure of growth traits:

Body weight at birth (BW) and weaning (WW) and daily weight gain (DG) were collected from the APRI database file of the six buffalo experimental herds (NG, NK, EN, ES, EG and S). Data on body weight were collected from 8229 buffalo calves, progeny of 277 sires and 2175 dams for a period of 22 years from 2003 to 2024. The numbers of calves and records in the pedigree and data files in different herds are shown in Table 1. All available relationships among animals were considered in the analyses.

Table 1. Number of Egyptian buffalo in the pedigree file used in genetic analyses for body weight and gain in six herds

Item	Herd						
	NK	NG	EN	EG	ES	S	All herds
Number of calves with records	1375	3591	95	2105	64	999	8229
Number of sires with records	49	123	24	48	8	25	277
Number of dams with records	329	917	39	562	41	287	2175
Total number of calves with records and sires and dams without records	1753	4631	158	2715	113	1311	10681

NG= El-Nattafe El-Gadid herd, NK= El-Nattafe El-Kadim herd, N= Nubaria herd, G= El-Gimmeza herd, ES= EL-Serw herd and S=Sids herd.

Animal model for analyzing data of growth traits:

Data of BW, WW and DG were evaluated fitting the fixed effects in the model to avoid over-parameterization. The variance components of random effects and heritabilities were estimated by TM software of a Bayesian Inference Gibbs Sampling Algorithm (Legarra *et al*., 2008). The estimates obtained from Gibbs sampling were used to solve the corresponding mixed model equations, obtaining the generalized least-square means (GLM) for BW, WW and DG using the PEST software (Groeneveld, 2006**)**. Then, the following single-trait animal model was used:

$$\mathbf{y = Xb + Z_a u_a + Z_c u_c + e} \quad \textbf{(Model 1)}$$

where **y** = the vector of observed BW or WW or DG of buffalo calves; **b** = the vector of fixed effects of herd-year-season of calf birth (382 levels), sex of calf (males or females), parity order (five levels); $\mathbf{u_a}$ = the vector of random additive genetic effects of the buffalo calves; $\mathbf{u_c}$ = the vector of random permanent environmental effects of dam; **X,** $\mathbf{Z_a}$ and $\mathbf{Z_c}$= the incidence matrices relating records to the fixed effects, additive genetic effects and random permanent environmental effects of dam, respectively; **e** = the vector of random residual effects. The heritabilities were estimated using the following equation: $\boldsymbol{h^2 = \frac{{\sigma^2}_a}{{\sigma^2}_a + {\sigma^2}_c + {\sigma^2}_e}}$, where ${\sigma^2}_a$ = the additive genetic variance for BW, WW and DG traits, ${\sigma^2}_c$ = the maternal permanent environmental variance and ${\sigma^2}_e$ = the error variance.

Predicting breeding values (PBVs) using BLUPF90 software:

The predicted breeding values and their predicted error variance (PEV, *i.e*. standard errors, SE) and accuracies of predictions (r_A) for BW, WW and DG of 10681 animals (calves with records and sires and dams without records) were estimated using the computer package of BLUPF90 software (Misztal *et al*., 2018). The values of PBV were estimated using the animal model previously mentioned in Model 1. The accuracy of predicted breeding values (r_A) was defined as the correlation coefficient between the true and predicted breeding value. The accuracy of PBV was calculated as described by Meyer (2004): $r_A = \sqrt{\mathbf{1} - (\boldsymbol{PEV}/\boldsymbol{\sigma}^2\mathbf{a})}$, where PEV= the prediction error variance estimated using elements from the mixed model equations as PEV = $(SEP)^2$ and SEP = the standard error of prediction and σ^2_a = the additive genetic variance of the trait.

Plotting the genetic and phenotypic trends:

The breeding values for growth traits of 10681 animals with and without records were estimated by BLUPF90 software (Misztal *et al*., 2018) and the PBV for calves with records and sires and dams without records were used in plotting the genetic trends by regressing the breeding values of body weights and gains on herd-year-season of birth of calves. The phenotypic trends were plotted by regressing the phenotypic values of BW, WW and DG for records of (8229 calves for BW, 8203 calves for WW and 8181 calves for DG) on herd-year-season of birth of calves (382 levels).

Animals and records used in molecular analyses:

Egyptian buffalo as a river buffalo have 50 chromosomes (25 pairs). A total of 286 (200 female and 86 male) blood samples were collected from buffalo calves randomly selected of the studied buffalo herds for genotyping. A total of 174 genotyped calves (with 174 records) from NG, NK and EG herds has been used for the molecular analyses of the candidate genes (*GH, PRL* and *FSHR*) associated with growth traits. All available relationships among calves were considered in molecular data analyses.

Blood sampling and DNA extraction:

For DNA extraction and amplification, blood samples were collected from the jugular veins of 200 female calves and 86 male calves in vacutainer tubes containing EDTA. All the samples were labeled, stored in an ice box and transferred to the laboratory and stored at -20ºC to be used in further processing. Genomic DNA was extracted from leukocytes using the QIAamp® Whole Blood Genomic DNA purity Kit (QIAGEN, Hilden, Germany). An amount of 20 *µl* of proteinase K solution was added to 200 *µl* of whole blood in 2 ml Eppendorf tube and mixed by overtaxing; then 200 *µl* of lysis solution was added and mixed thoroughly by pipetting to obtain a uniform suspension. The sample was incubated at 56ºC for 10 minutes using a shaking water bath until the cells were completely lysed. 200 *µl* ethanol (96–100%) was added to the sample and remixed by pulse-vortex for 15 seconds. The prepared mixture was transferred to the spin column, centrifuged at 6000 rpm for one minute at room temperature and then the collection tube containing the flow-through solution was removed. The spin column was placed into a new 2 *ml* collection tube; then 500 *µl* of wash buffer AW1 was added and centrifuged at 8000 rpm for one minute at room temperature. The flow-through solution was discarded and the column placed back into the collection tube. A 500 *µl* of wash buffer AW2 was added to the column and centrifuged at 14000 *rpm* for three minutes at room temperature. The collection tube was emptied and the purification column placed back into the tube and centrifuged at full speed for one minute. This step helps to eliminate the chance of possible Buffer AW2 carryover. The collection tube containing the flow-through solution was discarded, transferring the column to a sterile 1.5 *ml* micro centrifuge tube. A 200 *µl* Buffer AE or distilled water was added to the center of the column membrane to elute the genomic DNA, incubated for two minutes and centrifuged at 8000 *rpm* for one minute at room temperature (15–25°C). Genomic DNA was stored at -20ºC. Then, high quality purified and concentrated DNA products were obtained to be used directly in a variety of downstream applications.

Amplification by polymerase chain reaction (PCR):

The PCR technique was used to amplify *GH*, *PRL* and *FSHR* genes, PCR product processing was performed in 25 *µl* reaction mixtures, containing 1.5 *mM* $MgCl_2$, 200 *µM* dNTP mix, 5 *pmol* of each primer, 10 × PCR buffer, 1 U Taq DNA polymerase and 100 *ng* of genomic DNA. The primers used in the amplification process are given in Table 2.

A 211 bp fragment of *GH* gene was amplified using the following primer set forward 5´- GCTGCTC CTGAGGGCCCTTC - 3´ and reverse 5'-CATGACCCTCAGGTACGTCTC CG -3' (Konca and Akyuz, 2017). The thermal cycling conditions were composed of a pre-denaturation step at 94°C for five minutes, followed by 30 cycles of denaturation at 94 ºC for one minute, annealing at 62ºC for one minute and extension at 72ºC for one minute and then final extension at 72˚C for five minutes.

A 678 *bp* fragment of *PRL* gene was amplified using the following primer set forward 5′ - AGGTTAGGAGGATAG-3′ and reverse 5′ - TTAGTCAAGTTAGATACCG-3′ (Hasanain *et al*., 2017). The thermal cycling conditions were composed of a pre-denaturation step at 95°C for three minutes, followed by 35 cycles at 95°C for one minute, 50.6°C for 60 seconds, extension at 72°C for one minute and the final extension at 72°C for five minutes.

A 306 *bp* fragment of *FSHR* gene was amplified using the following primer set forward 5´-CTGCCTCCCTCAAGGTGCCCCTC-3′ and reverse 5'-AGTTCTTGGCTAAATGTCTTAGGGGG -3' (Fouda *et al*., 2021). The PCR reaction was conducted as following: PCR tubes containing the mixture were subjected to five minutes at 95 ºC for initial

denaturation, 30 cycles of amplification (denaturation at 95 ºC for thirty seconds, annealing at 60 ºC for thirty seconds and extension at 72 ºC for thirty seconds) and final extension at 72 ºC for eight minutes.

Aliquots of 10 *µl* of the PCR amplicons for *GH*, *PRL* and *FSHR* genes were electrophoresed using 2 % Ethidium Bromide agarose gel at constant voltage of 100 for 30 minutes, then visualized under UV light with a Gel Doc 1000 system (Bio-Rad).

Digestion and genotyping of GH, PRL and FSHR genes using PCR-RFLP technique:

To characterize *GH*, *PRL* and *FSHR* genes according to their restriction pattern, the PCR product of each gene was digested with the proper restriction enzyme, as specified in Table 2. Each enzymatic reaction consisted of a 25 *µl* mix including 0.5 *µl* (10u/*µl*) of restriction enzyme (Fermentas), 2.5*µl* of 10x NE Buffer, 5 *µl* of PCR product, 0.1 *mg/ml* acetylated Bovine serum albumin (BSA), and 16.75 *µl* of sterile dH2O. The digested fragments were visualized by electrophoresis on 2.5 % agarose gel at 120 V in 1xTAE. The 250 bp DNA step ladder (Promega) was included in each run. After electrophoresis, the gel was stained with ethidium bromide 0.5 µg/ml. Fragments were visualized a UV transilluminator and the images were digitalized by the Gel Doc^{TM}XR^{+}(BIO-RAD) gel documentation system. The PCR-RFLP technique was used in genotyping the SNP genotypes located in the promoter regions of these genes. Also, PCR-RFLP technique and *AluI* and *XbaI* restriction enzymes were used to detect the molecular associations between SNP genotypes of *GH, PRL* and *FSHR* candidate genes and body weights and gains traits in the Egyptian buffalo. Out of the 286 blood samples (200 female and 86 male) collected from the buffalo calves belonged to the NG, NK and EG herds, a total of 174 animals (about 61% of the total blood samples) were successfully genotyped using PCR-RFLP.

Table 2. Primer sequence and PCR-RFLPassay conditions for genotyping SNPs of *GH, PRL* and *FSHR* genes

Gene	CN^{+}	Primer sequences (forward/reverse)	PCR Product size (bp)	Annealing temp (ºC per time, s)	Restriction Enzyme
GH	3	5´- GCTGCTCCTGAGGGCCCTTC - 3´ 5'-CATGACCCTCAGGTACGTCTCCG -3'	211	62/60	*AluI*
PRL	2	5′ -AGGTTAGGAGGATAG-3′ 5′ -TTAGTCAAGTTAGATACCG-3′	678	50.6/60	*XbaI*
FSHR	12	5´-CTGCCTCCCTCAAGGTGCCCCTC-3′ 5'-AGTTCTTGGCTAAATGTCTTAGGGGG-3'	306	60/30	*AluI*

$^{+}$CN= Chromosome number.

Models for detecting the polymorphic associations:

The molecular associations among genotypes of *GH, PRL* and *FSHR* genes and body weights and gains were assessed using Model 1 after adding the SNP genotypes of *GH* gene (TC and CC genotypes) or *PRL* gene (AA and GG genotypes) or *FSHR* gene (GG, GC and CC genotypes) as fixed effects. The estimates obtained from Bayesian Gibbs Sampling Algorithm (Model 1) were used to solve the corresponding mixed model equations and obtain the generalized least-square means of body weights and gains for different genotypes using the PEST software (Groeneveld, 2006).

RESULTS AND DISCUSSION

Descriptive statistics, heritability estimates and maternal permanent environmental effects:

The GLM, standard deviations (SD), minimum and maximum values, coefficients of variation (CV %), heritability estimates and proportion of the maternal permanent environmental effects for BW, WW and DG are shown in Table (3). The GLM for BW, WW and DG were 35.0 *kg*, 94.7 *kg* and 0.616 *kg*, respectively. In other studies, on Egyptian buffalo, lower means of 33 *kg* for BW was reported by El-Awady *et al.* (2005), 87 *kg* for WW was reported by Ashmawy and El-Bramony (2017) and 32.78 *kg* for BW and 91.96 *kg* for WW by El-Den *et al*. (2020).

The ranges between minimum and maximum values of body weights and gains in the Egyptian buffalo in the present study were high, being 15 to 53 *kg* for BW, 50 to 147 *kg* for WW and 0.10 to 1.40 *kg* for DG, with coefficients of variation of 18, 13 and 24 % for BW, WW and DG, respectively (Table 3). In this respect, moderate or high coefficient of variation was reported by Easa *et al*. (2022) for BW in the Egyptian buffalo (15.5%), while was 23.0 % for WW of the Colombian buffalo (Agudelo-Gómez *et al*., 2015).

The heritability values estimated by animal model for BW, WW and DG were mostly moderate or high, being 0.26, 0.50 and 0.55, respectively (Table 3), indicating that the Egyptian buffalo herds in the present study were not subjected to intensive programmes of selection. Therefore, there is a future possibility for successful direct selection on body weights and gains in the studied buffalo populations. However, the heritability estimates for BW and WW were mostly similar to these estimates cited in the Egyptian buffalo studies (EL-Awady *et al*., 2005; Shahin *et al*., 2010; Ashmawy and El-Bramony, 2017; El-Sayed *et al*., 2020; El-Den *et al*., 2020; Salem *et al*., 2021 and Easa *et al*., 2022) and in the Murrah and Nili-Ravi buffalo studies in Brazil, India, Pakistan, Colombia and Italy (Cassiano *et al*., 2004; Suhail *et al*., 2009; Malhado *et al*., 2012; Gupta *et al*., 2015; Agudelo-Gómez *et al*., 2015 and Rezende *et al*., 2020). Differences among estimated and reviewed heritabilities may be attributed to the structure and

genetic variation of the studied populations, method of variance components estimation and model of analysis (Malhado *et al*, 2012) and environmental deviations, large standard errors due to small datasets as well as to the fact that body weights and gains are strongly influenced by the management scheme and due to possible variation in seasonal supply of green feedstuffs. The proportions of maternal permanent environmental effects (c^2) were moderate for BW, WW and DG, being 0.23, 0.34 and 0.24, respectively (Table 3). The variation in WW due to maternal permanent environmental effects was also moderate but higher than the value for BW, indicating that the permanent environmental influence of the buffalo dam has considerable maternal carry over environmental effects on calves from birth to weaning, *i.e.* the maternal environmental effects of buffalo dams were dominant from birth until weaning. In this regard, Cassiano *et al.* (2004) reported that the maternal permanent environment effects for birth weight were low or medium being 0.11, 0.17, 0.37 and 0.04 for Carabao, Jaffarabadi, Mediterranean and Murrah buffalo, respectively. Malhado *et al.* (2007) showed that the c^2 for body weight at 205 days were high (0.43) in Brazilian buffalo.

Table 3. Descriptive statistics, heritability estimates (h^2), proportions of maternal permanent environmental effects (c^2) and random error effects (e^2) for growth traits of Egyptian buffalo

Item	BW (*kg*)	WW (*kg*)	DG (*kg*)
Descriptive statistics[+]:			
Numbers of values	8229	8203	8181
GLM	35.0	94.7	0.616
SD	6.32	12.3	0.151
Minimum value	15	50	0.10
Maximum value	53	147	1.40
Coefficient of variation (CV %)	18	13	24
Heritability estimates and maternal permanent environmental effects estimated by Single-trait Animal Model:			
h^2±SE	0.26±0.036	0.50±0.016	0.55±0.019
c^2±SE	0.23±0.008	0.34±0.015	0.24±0.014
e^2±SE	0.51±0.035	0.12±0.013	0.19±0.018

BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.
[+]GLM= Generalized least square means estimated by Animal Model using PEST software, SD= standard deviations, SE=Stander error.

Predicted breeding values (PBVs):

The estimates of minimum and maximum PBV and their accuracies of predictions (r_A) for BW, WW and DG are given in Table (4). Wide variations in PBVs of 10681 animals were observed, ranging from -4.2 to 3.5 *kg* for BW, -42.4 to 44.2 *kg* for WW and -0.44 to 0.52 *kg* for DG. The percentages of animals with positive PBVs (buffalo calves with records and sires and dams without records) for body weights and gains were high ranging from 54 to 59 % (Table 4). Thus, the high genetic variabilities in body weights and gains indicated that there are good opportunities to improve these traits in Egyptian buffalo through selection. Similar wide variations in PBVs were observed in some buffalo studies (EL-Awady *et al*., 2005 and Agudelo-Gómez *et al*., 2015). In the Egyptian buffaloes, EL-Awady *et al*. (2005) found that the ranges in PBVs for calves were high ranging from -4.8 to 3.4 *kg* for BW, -15.8 to 9.7 *kg* for WW and -131 to 99 *g* for DG, associated with high ranges in PBVs for sires (-2.3 to 2.6 *kg* for BW, -6.4 to 15.5 *kg* for WW and -79.9 to 116 *g* for DG) and also high ranges in PBVs for buffalo dams (-2.9 to 2.1 *kg* for BW, -10.6 to 15.5 *kg* for WW and -111 to 118 *g* for DG). On the contrary, El-Sayed *et al.* (2020) and Salem *et al.* (2021) reported that the ranges in PBVs were low and ranged from -0.02 to 0.2 *kg* for BW and -0.02 to 0.5 *kg* for WW.

The accuracies (r_A) of minimum and maximum PBVs for body weights or gains were high, ranging from 0.63 to 0.89 (Table 4). These high accuracies may be due to that heritabilities for body weights and gains were highly associated with more available pedigree information for the studied buffalo calves along with their sires and dams (EL-Awady *et al*., 2005; El-Sayed *et al.*, 2020 and Salem *et al.*, 2021). However, high accuracies in PBVs obtained in the present study indicated that selection of the buffalo calves in these herds could be used as parents in the next generations, and this would lead to sustainable genetic improvement for growth traits in Egyptian buffalo.

Table 4. Minimum and maximum predicted breeding values (PBV), their standard errors (SE) and accuracies of predictions (r_A) for growth traits

Trait	No. of animals	Minimum PBV	SE	r_A	Maximum PBV	SE	r_A	Range in PBV	Positive PBV (%)
BW (*kg*)	10681	-4.2	1.69	0.65	3.5	1.73	0.63	7.70	59
WW (*kg*)	10681	-42.4	4.47	0.89	44.2	4.86	0.88	86.6	54
DG (*kg*)	10681	-0.44	0.065	0.84	0.52	0.07	0.83	0.96	56

SE=Stander error; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.

Genetic and phenotypic trends:

The genetic trends plotted for BW, WW and DG across the years from 2003 to 2024 are shown in Figure 1. The regression line of PBVs for body weights and gains of 10681 animals are showing slight increase in the genetic trends as year-season of calving advanced, the ranges increased slightly from 1.6 to 1.8 *kg* for BW, -0.519 to 1.57 *kg* for WW and -24 to 18 *g* for DG. Gupta *et al*. (2015) showed that genetic trend for WW in Murrah buffalo increased as year of calving advanced. But the wide ranges in the genetic trends (Figure 1) reflected precise methodology of culling and replacement processes performed in the studied herds. However, the slight increase in genetic trends registered in the present study over 22 years period can be justified by the facts that: 1) Progeny testing of selection was not practiced properly or not performed on a large scale, 2) Selection towards the desired changes over 22 years was not effective enough due to the lack of efficient selection or breeding methods to evaluate the calves, 3) Herds size were small, 4) Inbreeding was practiced in few cases, 5) lack of accuracy in performance recording, 6) Few elite sires were used in the breeding strategy in the recent years, 7) Small set of random mating was practiced in some small herds, and 8) Young calves were selected on the basis of growth rate without considering their breeding values.

The phenotypic trends plotted for body weights and gains during the period from 2003 to 2024 are shown in Figure 2. The ranges in phenotypic values for BW (8229 calves), WW (8203 calves) and DG (8181 calves) showed non-favorable decreasing in values of the phenotypic trend as year-season of calving advanced. The ranges in phenotypic values of herd-year-season of calving for body weights and gains decreased slightly from 36.6 to 32.9 *kg* for BW, 94.55 to 94.15 *kg* for WW and 628 to 582 *g* for DG. This slight decrease in phenotypic trends of all weights and gains may be attributed to low nutritional and feeding levels applied and unsuitable management schemes practiced in different herds. In the buffalo literature, the genetic and phenotypic trends for BW and WW were favorable showing an increase in body weights and gains as stated in Brazilian buffalo (Malhado *et al*., 2007), in Murrah buffalo (Gupta *et al*., 2015) and in Egyptian buffalo **(**El-Bramony, 2014; El-sayed *et al*., 2020 and Salem *et al*., 2021).

Molecular associations among GH gene or PRL gene and growth traits:

The molecular association analyses for growth traits revealed that two genotypes (Dimorphic) of TC and CC were detected for *GH* gene in each separate herd. The associations were significantly in favour of TC genotype in NG, NK and EG herds ($P<0.01$, Table 5). Across all herds, GLM for TC genotype had significantly heavier body weights and gains than CC genotype (36.8 *vs* 33.9 *kg* for BW, 96.3 *vs* 91.8 *kg* for WW and 600 *vs* 540 *g* for DG). For each experimental herd, the GLM of BW, WW and DG of TC genotype were favorably higher relative to CC genotype (36.9 *kg*, 94.8 *kg* and 590 *g vs* 34.4 *kg*, 91.2 *kg* and 560 *g* in NG herd; 38.0 *kg*, 95.9 *kg* and 580 *g vs* 36.5 *kg*, 90.9 *kg* and 530 *g* in NK herd; 39.0 *kg*, 104.6 *kg* and 660 *g vs* 33.0 *kg*, 91.5 *kg* and 530 *g* in EG herd). Studies on Egyptian buffalo have shown that *GH* gene can be used as a candidate gene for the genetic improvement of growth traits **(**Othman *et al*., 2012a and Darwish *et al*., 2016**)**. Studies on non-Egyptian buffalo have shown polymorphic associations between *GH* candidate gene and growth and carcass traits in Indonesian buffalo (Andreas *et al*., 2010; Eriani *et al*., 2019 and Nafiu *et al*., 2020) and Anatolian buffalo in Turkey (Konca and Akyüz, 2017 and Özkan *et al*., 2020).

Regarding the *PRL* gene, the molecular association analyses showed that two genotypes (Dimorphic) AA and GG for *PRL* gene were identified in each herd (NG, NK and EG; Table 5). Across all herds, GLM for AA genotype was significantly heavier in body weights and gains ($P<0.01$) than GG genotype (38.6 *vs* 36.1 *kg* for BW, 93.7 *vs* 90.8 *kg* for WW and 594 *vs* 568 *g* for DG). Similarly, the GLM in each separate herd for BW, WW and DG in AA genotype were favorably higher in weights and gains relative to GG genotype (43.9 *kg*, 95.9 *kg* and 594 *g vs* 33.9 *kg*, 92.6 *kg* and 472 *g* in NG herd; 36.4 *kg*, 95.0 *kg* and 605 *g vs* 34.0 *kg*, 90.8 *kg* and 542 *g* in NK herd; 43.7 *kg*, 99.9 *kg* and 623 *g vs* 34.8 *kg*, 77.6 *kg* and 367 *g* in EG herd). To our knowledge, there are no previous studies concerning the molecular association between *PRL* gene and growth traits in buffalo although there are limited studies available in cattle. Meyer *et al*. (2017) demonstrated that genotypes of *PRL* gene impacted significantly heavier live body weights of Angus calves at birth and weaning.

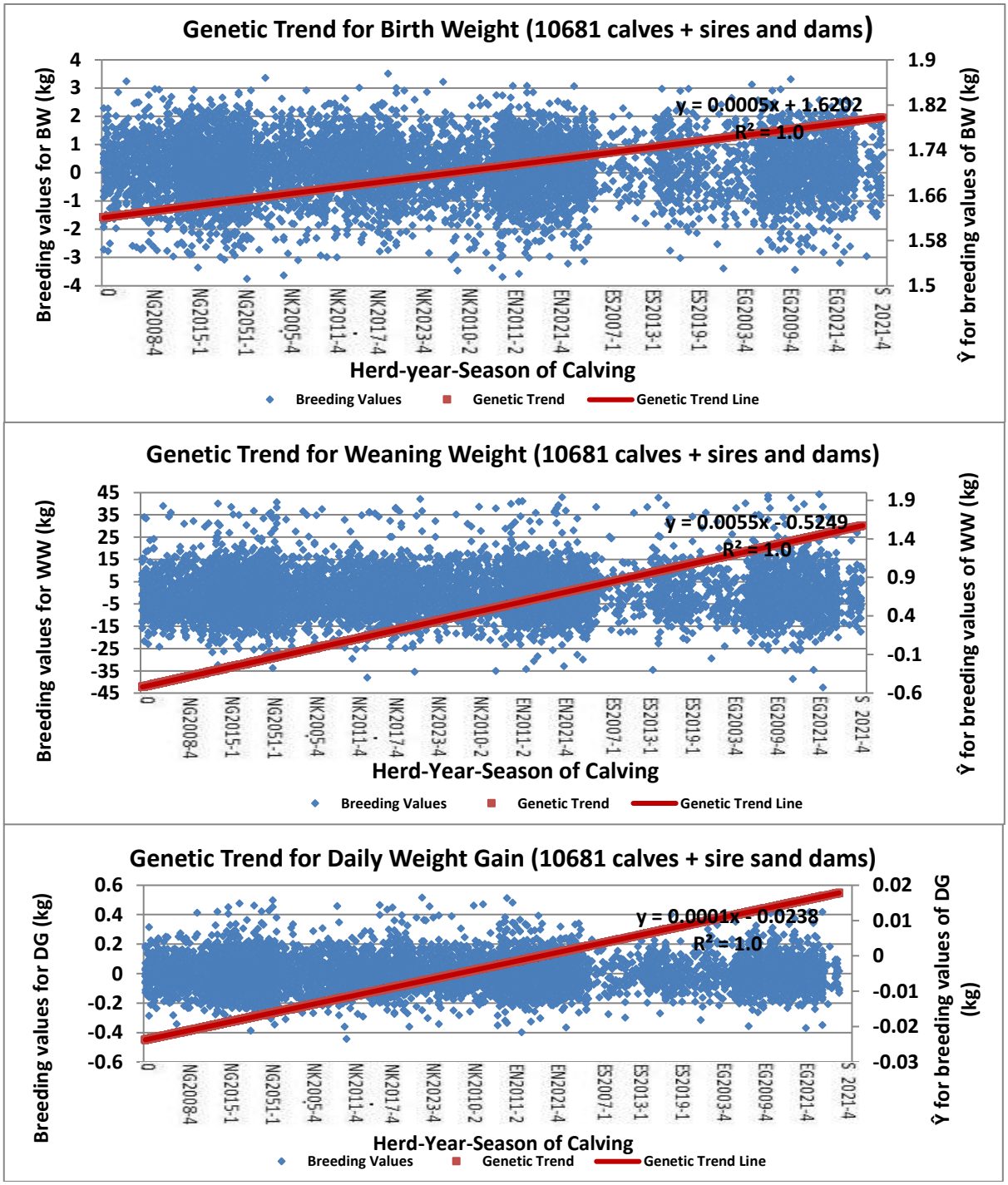

Figure 1. Genetic trends for birth weight (BW), weaning weight (WW) and daily weight gain (DG) in Egyptian buffalo plotted by regressing the breeding values of growth trait on herd-year season of calving in El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S) herds.

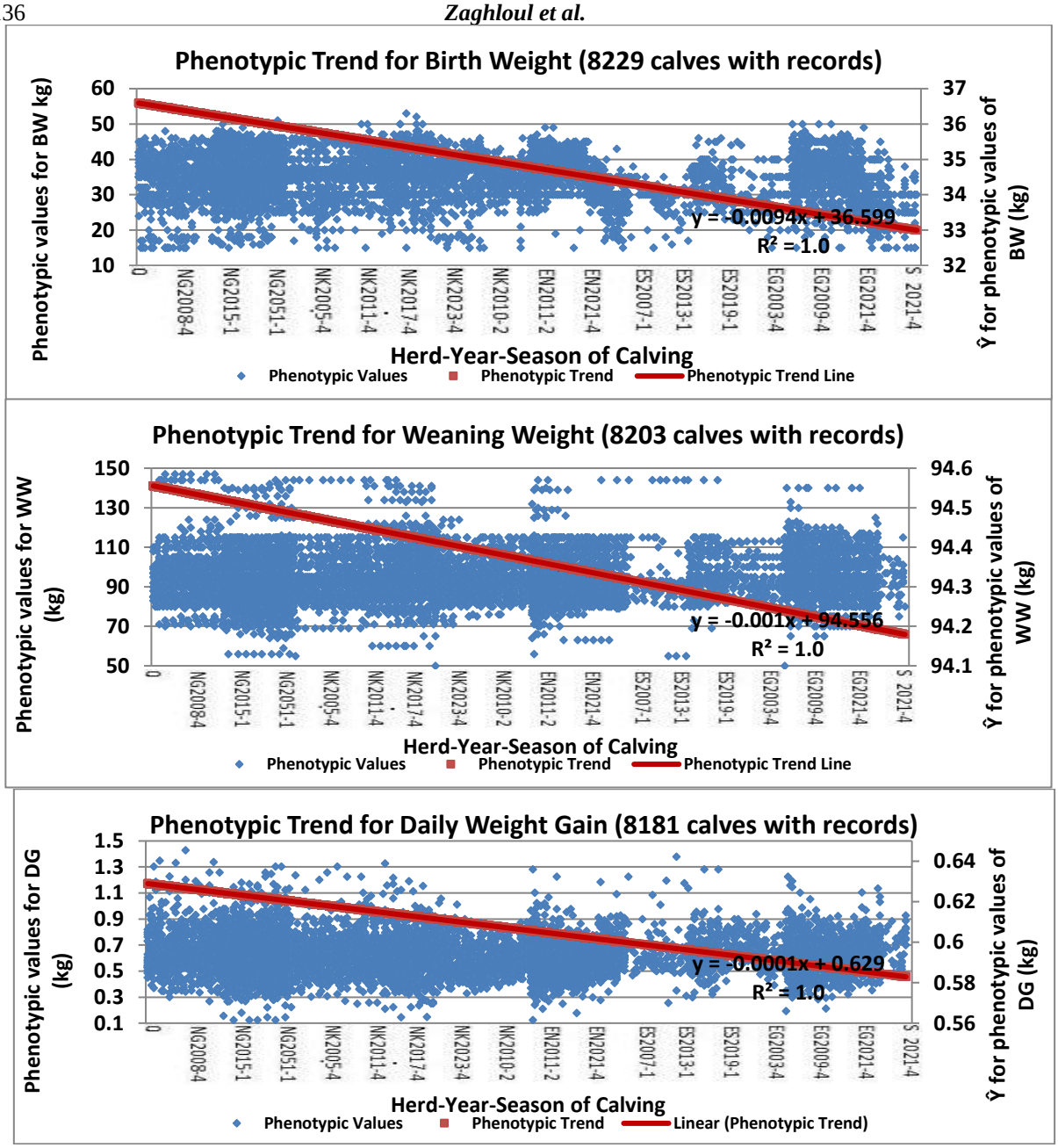

Figure 2. Phenotypic trends for birth weight (BW), weaning weight (WW) and daily weight gain (DG)in Egyptian buffalo plotted by regressing the phenotypic values of growth traits on herd-year season of calvingin El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S) herds.

Molecular associations between FSHR gene and growth traits:

The molecular association analyses revealed that three genotypes (Polymorphic) of GG, GC and CC for *FSHR* gene were detected (Table 6). The differences in GLM among GG, GC and CC genotypes of *FSHR* gene for BW, WW and DG were significantly in favour of GG relative to GC and CC genotypes (P<0.01). Across all herds, the GLM for GG genotype were significantly heavier in weights than GC and CC genotypes (38.8 *kg vs* 35.4 and 36.8 *kg* for BW; 97.9 *kg vs* 92.8 and 92.6 *kg* for WW; 603 *g vs* 558 and 452 *g* for DG). In each of NG and NK herds, GLM of GG genotype for BW, WW and DG were favorably heavier in weighs and gains relative to GC and CC genotype (37.7 *kg*, 102.9 *kg* and 662 *g* for GG genotype *vs* 32.9 *kg*, 89.1 *kg* and 550 *g* for GC genotype and 36.3 *kg*, 88.8 *kg* and 524 *g* for CC genotype in NG herd; 38.1 *kg*, 95.3 *kg* and 577 *g* for GG genotype *vs* 36.5 *kg*, 92.8 *kg* and 552 *g* for GC genotype and 36.5 *kg*, 92.5 *kg* and 537 *g* for CC genotype in NK herd). Oppositely, GLM for BW, WW and DG of CC genotype in EG herd were significantly heavier than GG and GC genotypes (39.4 *kg*, 106.2 *kg* and 636 *g* for CC genotype *vs* 33.3 *kg*, 93.3 *kg* and 574 *g* for GC genotype and 35.5 *kg*, 92.5 *kg* and 548 *g* for GG genotype). The reverse trend of association between the SNP genotypes of

FSHR gene and growth traits obtained in the EG herd could be attributable to the small number of the genotyped animals (24 calves) compared with the other two herds (47 calves of NG and 101 calves of NK herds). Therefore, more genotyped animals are required to represent the sample of the buffalo population raised in the EG herd. To the best of our knowledge, there have been no anterior studies either on buffalo or on cattle assessing the molecular relationship between the *PRL* gene and growth traits.

Table 5. Molecular associations between *GH* gene or *PRL* gene and growth traits in the Egyptian buffalo expressed as generalized least square means and their standard errors (GLM±SE)

Herd and growth trait	*GH* gene				Herd and growth trait	*PRL* gene			
	TC Genotype		CC Genotype			AA Genotype		GG Genotype	
	GLM	SE	GLM	SE		GLM	SE	GLM	SE
NG herd (N=51):	(N=20)		(N=31)		**NG herd (N=33):**	(N=29)		(N=4)	
BW (*kg*)	36.9[a]	1.3	34.4[b]	1.08	BW (kg)	43.9[a]	1.36	33.9[b]	3.67
WW (*kg*)	94.8[a]	2.2	91.2[b]	1.80	WW (kg)	95.9[a]	1.96	92.6[b]	5.28
DG (*g*)	590[a]	12	560[b]	22	DG (g)	594[a]	26	472[b]	71
NK herd (N=70):	(N=23)		(N=47)		**NK herd (N=45):**	(N=37)		(N=8)	
BW (*kg*)	38.0[a]	0.6	36.5[b]	0.67	BW (kg)	36.4[a]	0.90	34.0[b]	1.94
WW (*kg*)	95.9[a]	1.6	90.9[b]	1.62	WW (kg)	95.0[a]	1.85	90.8[b]	3.97
DG (*g*)	580[a]	20	530[b]	19	DG (g)	605[a]	19	542[b]	40
EG herd (N=53):	(N=40)		(N=13)		**EG herd (N=23):**	(N=20)		(N=3)	
BW (*kg*)	39.0[a]	1.3	33.0[b]	1.31	BW (kg)	43.7[a]	0.99	34.8[b]	2.56
WW (*kg*)	104.6[a]	3.3	91.5[b]	3.27	WW (kg)	99.9[a]	2.66	77.6[b]	6.87
DG (*g*)	660[a]	20	530[b]	34	DG (g)	623[a]	26	367[b]	68
All herds (N=174):	(N=83)		(N=91)		**All herds (N=101):**	(N=86)		(N=15)	
BW (*kg*)	36.8[a]	0.5	33.9[b]	0.53	BW (kg)	38.6[a]	0.63	36.1[b]	1.52
WW (*kg*)	96.3[a]	1.2	91.8[b]	1.18	WW (kg)	93.7[a]	1.2	90.8[b]	2.93
DG (*g*)	600[a]	12	540[b]	13	DG (g)	594[a]	13	568[b]	32

N= Number of records; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.
[a,b]GLM within each classification, not followed by the same letter in the row differed significantly (P<0.01).

Table 6. Molecular associations between *FSHR* gene and growth traits in Egyptian buffalo expressed as generalized least square means and their standard errors (GLM±SE)

Herd and growth trait	GG Genotype		GC Genotype		CC Genotype	
	GLM	SE	GLM	SE	GLM	SE
NG herd (N=47)	(N=10)		(N=23)		(N=14)	
BW (*kg*)	37.7[a]	1.92	32.9[c]	1.27	36.3[b]	1.63
WW (*kg*)	102.9[a]	3.21	89.1[b]	2.12	88.8[b]	2.71
DG (*g*)	662[a]	39	550[b]	25	524[b]	33
NK herd (N=101)	(N=22)		(N=38)		(N=41)	
BW (*kg*)	38.1[a]	0.85	36.5[b]	0.65	36.5[b]	0.62
WW (*kg*)	95.3[a]	2.83	92.8[b]	1.90	92.5[b]	1.83
DG (*g*)	577[a]	28	552[a]	21	537[a]	20
EG herd (N=24)	(N=3)		(N=11)		(N=10)	
BW (*kg*)	36.5[b]	2.67	33.3[c]	1.39	39.4[a]	1.46
WW (*kg*)	92.5[b]	6.42	93.3[b]	3.35	106.2[a]	3.52
DG (*g*)	548[b]	69	574[b]	36	636[a]	37
All herds (N=172)	(N=35)		(N=72)		(N=65)	
BW (*kg*)	38.8[a]	0.55	35.4[c]	0.79	36.8[b]	0.57
WW (*kg*)	97.9[a]	1.93	92.8[b]	1.35	92.6[b]	1.40
DG (*g*)	603[a]	21	558[b]	15	452[b]	15

N= Number of growth traits records; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.
[a,b]GLM within each classification, not followed by the same letter in the row differed significantly (P<0.01).

CONCLUSIONS

Birth or weaning weight could be adopted as an early selection criterion to improve growth performance in Egyptian buffalo. Improving management and feeding schemes and using accurate estimates of predicted breeding values in the genetic improvement programs, should improve growth traits in Egyptian buffaloes efficiently. Based on the significant molecular associations detected in the present study, TC genotype of *GH* gene, AA genotype of *PRL* gene and GG genotype of *FSHR* gene could be used as advantageous marker-assisted tools in selection programs, aiming to improve growth traits in Egyptian buffalo.

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ETHICS STATEMENT

All experimental procedures involving animals handling and treatment were approved by the Research Ethics Committee of the Faculty of Agriculture, Benha University, Egypt (Approval No REC-FOABU. 3/00021).

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الإتجاهات الوراثية والمظهرية لأوزان الميلاد والفطام والإرتباطات الجزيئية لهذه الأوزان مع جينات *GH*، *PRL*، *F* *SHR* في الجاموس المصري

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إستهدفت هذه الدراسة التقييم الوراثي لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن من الميلاد حتي الفطام في الجاموس المصري وذلك عن طريق تقدير مكونات التباين والمكافئ الوراثي بإستخدام نموذج الحيوان Animal Model (AM) معتمداً على أسلوب Bayesian Gibbs Sampling Algorithm وكذا تقدير القيم التربوية للحيوانات (PBVs) ورسم خطوط الإتجاهات الوراثية والمظهرية لصفات النمو بإستخدام برنامج BLUPF90، وكذلك الكشف عن الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *GH*، *PRL*، *FSHR* وصفات النمو وذلك بإستخدام تقنية PCR-RFLP. إستخدمت البيانات التي تم تسجيلها للوزن عند الميلاد والفطام لعدد ٨٢٢٩ من عجول الجاموس الناتجة من ٢٧٧ أب، ٢١٧٥ أم في ستة قطعان للجاموس المصري (النطاف الجديد، النطاف القديم، النوبارية، السرو، الجميزة، سدس) التابعة لمعهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، وزارة الزراعة، مصر. بينما إستخدمت بيانات ثلاثة قطعان (النطاف الجديد، النطاف القديم، الجميزة) لعدد ١٧٤ حيوان في تحليلات الإرتباطات الجزيئية بين التراكيب الوراثية للجينات وصفات النمو. كانت قيم المكافئ الوراثي المحسوبة من نموذج الحيوان Animal Model متوسطة إلى مرتفعة لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن من الميلاد حتى الفطام حيث كانت القيم ٠,٢٦، ٠,٥٠، ٠,٥٥ على الترتيب. تراوحت القيم التربوية بين -٤,٢ إلى ٣,٥ كجم لصفة الوزن عند الميلاد، -٤٢,٤ إلى ٤٤,٢ كجم لصفة الوزن عند الفطام، -٠,٤٤ إلى ٠,٥٢ كجم لصفة معدل الزيادة اليومية في الوزن. في حين إرتفعت قيم الإتجاهات الوراثية لوزن الجسم ومعدل الزيادة اليومية إرتفاعاً معنوياً من ١,٦ كجم لصفة الوزن عند الميلاد ومن -٠,٥١٩ كجم ليصبح ١,٥٧ كجم لصفة الوزن عند الفطام ومن -٢٤ جرام ليصبح ١٨ جرام لصفة معدل الزيادة اليومية في الوزن. بينما أظهرت الإتجاهات المظهرية تدهوراً تراوحت قيمته من ٣٦,٦ كجم ليصبح ٣٢,٩ كجم لصفة الوزن عند الميلاد ومن ٩٤,٥٥ كجم ليصبح ٩٤,١٥ كجم لصفة الوزن عند الفطام ومن ٦٢٨ جرام ليصبح ٥٨٢ جرام لصفة معدل الزيادة اليومية في الوزن. بإستخدام تقنية PCR-RFLP في قطعان النطاف الجديد والنطاف القديم والجميزة تم الكشف عن تركيبين وراثيين هم TC،CC لجين *GH* (ثنائي النمط) وكانت متوسطات المربعات الصغري المعممة لصفات النمو معنوية وفي صالح التركيب الوراثي TC مقارنة بالتركيب الواثي CC (٣٦,٩ كجم، ٩٤,٨ كجم، ٥٩٠ جرام مقابل ٣٤,٤ كجم، ٩١,٢ كجم، ٥٦٠ جرام في قطيع النطاف الجديد وكذلك ٣٨,٠ كجم، ٩٥,٩ كجم، ٥٨٠ جرام مقابل ٣٦,٥ كجم، ٩٠,٩ كجم، ٥٣٠ جرام في قطيع النطاف القديم، ٣٩,٠ كجم، ١٠٤,٦ كجم، ٦٦٠ جرام مقابل ٣٣,٠ كجم، ٩١,٥ كجم، ٥٣٠ جرام في قطيع الجميزة لصفات الوزن عند الميلاد، الوزن عند الفطام، ومعدل الزيادة اليومية في الوزن على الترتيب). كما تم الكشف عن تركيبين وراثيين هما AA، GG لجين البرولاكتين *PRL* (ثنائي النمط) في كل قطيع على حده، حيث كانت متوسطات المربعات الصغري المعممة لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن معنوية وفي صالح التركيب الوراثي AA مقارنة بالتركيب الوراثي GG (٤٣,٩ كجم، ٩٥,٩ كجم، ٥٩٤ جرام مقابل ٣٣,٩ كجم، ٩٢,٦ كجم، ٤٧٢ جرام في قطيع النطاف الجديد، ٣٦,٤ كجم، ٩٥,٠ كجم، ٦٠٥ جرام مقابل ٣٤,٠ كجم، ٩٠,٨ كجم، ٥٤٢ جرام في قطيع النطاف القديم، ٤٣,٧ كجم، ٩٩,٩ كجم، ٦٢٣ جرام مقابل ٣٤,٨ كجم، ٧٧,٦ كجم، ٣٦٧ جرام في قطيع الجميزة). بينما تم الكشف عن ثلاث تراكيب وراثية هي GG، GC ، CC لجين *FSHR* (متعدد الأنماط) حيث كانت متوسطات المربعات الصغري المعممة لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن في الغالب لصالح التركيب الوراثي GG مقارنة بالتراكيب الوراثية CC، GC في قطعان النطاف الجديد والنطاف القديم (٣٧,٧ كجم، ١٠٢,٩ كجم، ٦٦٢ جرام للتركيب الوراثيGG مقابل ٣٢,٩ كجم، ٨٩,١ كجم، ٥٥٠ جرام للتركيب الوراثي GC وكذا ٣٦,٣ كجم، ٨٨,٨ كجم، ٥٢٤ جرام للتركيب الوراثي CC في قطيع النطاف الجديد، بينما كانت متوسطات المربعات الصغري المعممة ٣٨,١ كجم، ٩٥,٣ كجم، ٥٧٧ جرام للتركيب الوراثي GG مقابل ٣٦,٥ كجم، ٩٢,٨ كجم، ٥٥٢ جرام للتركيب الوراثي GC وكذا ٣٦,٥ كجم، ٩٢,٥ كجم، ٥٣٧ جرام للتركيب الوراثي CC في قطيع النطاف القديم). خلصت الدراسة تطبيقياً أنه من خلال المقاييس الوراثية المتحصل عليها (القيم التربوية المحتملة وخطوط الإتجاهات الوراثية والمظهرية) بالإضافة إلى الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *GH*، *PRL*، *FSHR* وصفات النمو أنه يمكننا إستخدام هذه العناصر بكفاءة في تقييم خطط التربية وبرامج التحسين الوراثي لتحسين آداء النمو في الجاموس المصري.