Analysis of rabbit growth hormone gene polymorphisms to evaluate its impact on live body weight, hemato and biochemical parameters

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ABSTRACT

Hormone (GH) promotes the growth and metabolism in mammals. Polymorphisms of this gene associated with production traits have been studied in many livestock. The objective of this study was to investigate the polymorphism in the part of growth intron 1, exon 2 and part of intron 2 of the rabbit GH gene and evaluate its effect on salutary body weight, some blood hemato and biochemical parameters. Therefore, twenty four rabbits consisting of 12 ♀ and 12♂ were randomly selected from each line to collect blood samples for DNA extraction and analysis of hemato and biochemical parameters. Specific –PCR was performed to amplify 345bp fragment of GH gene. Genotyping of the gene was carried out using Single Stranded Conformational Polymorphism (SSCP). The results revealed that two alleles (A and B) with AA and AB genotypes found in the two populations. The most frequent genotype was AB in Alexandria line while AA genotype was the predominant in V line. GH locus was more informative in Alexandria than V line. AB genotype showed higher significant differences as compared with AA ones in the most studied traits. The males and Alexandria line always exhibited higher score in the most traits in compareison with females and V line, respectively. The above results were found to be associated with histological examination data as jejunum villi, crypt depth and intestinal musculosa were significantly increased with AB genotype and Alex line. So, Screening of rabbit GH gene by PCR-SSCP for evaluation of its polymorphism with some economic traits may be useful to apply this gene as a marker-assisted selection in rabbit breeding programs.

Keywords: *GH gene, SSCP-PCR, polymorphism, hemato and biochemical parameters, rabbits*

INTRODUCTION

The domestic rabbit is characterized by early sexual maturity, high rate of reproduction, rapid growth rate, more efficient feed conversion and its profitability for small-scale system of production and land space utilization. Rabbit meat has a low cholesterol level and high ratio of protein. It is relatively rich in essential fatty acids so, its meat is still more economical in terms of feed energy than beef. These potentialities of rabbit have been considered as a good alternative source of animal protein and could contribute significantly in solving the problem of meat shortage (Lebas and Collin, 1992). Industry of rabbits is not widely spread as that for broiler or egg production industries in Egypt. In

addition to that, there is a less information in the genetic and managerial aspects of rabbit's production to create a profitable industry in Egypt. The First International Conference of Rabbit Production in Hot Climates held in Cairo in 1994 pointed out to start selection programs for improving the productivity of rabbit under local conditions (Youssef *et al*., 2008).This improvement requires the understanding of the genomic information of such complex traits whereas;genomic resources for the rabbit are still limited compared to other farm animal species.

A quantitative trait locus (QTL) is a section of DNA that correlates with variation in a phenotype. QTLs are mapped by identifying molecular marker genes that associates with an observed trait (Rothschild and Soller, 1997). Growth hormone (*GH)* gene can be considered as a candidate gene for growth and meat production in animals. It has a direct effect on the synthesis and secretion of growth hormone which modulates protein, carbohydrate and lipid metabolism in many tissues of animals. GH regulates the process of reproduction by controlling proliferation, apoptosis, growth and differentiation of several reproductive organs (Fontanesi *et al*., 2008 and Sirotkin, 2005) .Specific regions of the *GH* gene were analyzed to provide additional information for improving breeding programs in several animals. Few studies identified polymorphisms of the *GH* gene in rabbit and evaluated its effect association with complex traits (Fontanesi *et al*., 2012). Single strand Conformation Polymorphism (SSCP) is simple and easy molecular technique that is widely used for the detection of sequence variations at multiple positions in DNA fragments including point and insertion/deletion mutations under non-denaturing conditions through electrophoretic mobility differences .It can detect a PCR fragment which was in the range of 175-250 bp (Sunnucks *et al.,* 2000). Therefore, the objective of this paper is to introduce a simple, less expensive and high throughput method for amplification and detection of DNA marker representing in the part of intron1, exon2 and part of intron2 of the rabbit GH gene and study the polymorphisms in this segment of DNA in addition, evaluate its effect on live body weight (LBW) at 63 days of age and some physiological traits under Egyptian condition. Blood hematological parameters were also investigated to identify a suitable genotype that may help for improving rabbit breeding in the future. Effect of sex and lines on the above traits were also studied that could be useful for selection of these traits in rabbit lines. Histological characteristic in jejunum of small intestine of male and female rabbit lines was performed that may useful for supporting the present study.

MATERIALS AND METHODS

The current study was conducted in cooperation with genetics Dept., Poultry Dept., Faculty of Agriculture, Alexandria University and Nucleic Acid Research Dept., Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Animals Experiment

Animals experiment was carried out during the production season of 2014/2015 in nucleus breeding rabbit unit of the poultry Research Center, Poultry department, Faculty of Agriculture, Alexandria University, Egypt.

Table (1): Genotype, allele frequencies and genetic characteristic of rabbit growth hormone (GH) gene in V and Alexandria rabbit lines at 63 days of age .

Primer	Rabbit lines	Genotype frequency		Allele frequency		Genetic characteristic	
		AA	AB	A	B	He	PIC
GH (345bp)	V	0.72	0.32	0.83	0.17	0.282	0.242
	Alex	0.08	0.92	0.54	0.46	0.497	0.373

Where, He = Heterozygosity and PIC (Polymorphism Information Content) = (0.25< PIC < 0).

Rabbit lines

Spanish line (V Line): It is a synthetic maternal line imported to Egypt from Spain where it has been selected for litter size at weaning from four specialized maternal lines since 1981 at the Dept. of Animal Science of the Universidad Politecnica de Valencia, Valencia, Spain. Compared to other exotic breeds, it has revealed more productivity and number weaning that was taken as a criterion of selection in this line (Baselga, 2002). Alexandria line (Alex line): It is a new synthetic paternal rabbit line that originated from crossing Baladi Black bucks with V line does. It was established and developed at the nucleus breeding rabbit unit of the poultry research center, Faculty of Agriculture, Alexandria University. The criterion of selection in this line is daily weight gain from weaning to slaughter age (El-Raffa, 2010). Thirty high productive does from each line were chosen as they gave almost the same number of bunnies (8 -10) at birth, number of weaned rabbits (6 -7) per doe and bunnies of weight ranged 520-560 g. From their offspring, a total number of 220 unsexed weaned rabbit of V Line with an average of 7.3 rabbits/ doe and 190 unsexed weaned rabbit of Alex line with an average of 6.3 rabbits/ doe were selected at 28 days of age with an average initial weight (540 ± 20 g) and were left to be reared to 63 days of age (Slaughter age).

Housing and flock management

During the period of experiment, animals were housed in a windowed rabbitry; both flocks (Alex and V lines) were kept weighted and ear-numbered individually then transferred to the progeny cages in groups. Managerial, feeding, watering, hygienic and environmental conditions were kept the same for both flocks. The animals were subjected to a lighting period of 14-16 hours provided with natural ventilation and temperature. Water ad- libitum from nipple drinkers and food were provided to the animals during the experimental period. The flocks were fed on commercial pellet diet that contains 17.85% crude protein, 11.89% crude fiber, 2.75% fat and 2556 kcal /kg metabolic energy (ME).

Data collected live body weight and hemato-biochemical parameters

At the end of the experimental period, 24 rabbits at the age of 63 days from each line (12 ♀ and 12♂) were randomly chosen and weighted individually from each line then slaughtered. About 3 ml blood samples from each slaughtered rabbit was taken and collected into a clean sterilized tube with an anticoagulant disodium EDTA for estimating blood picture. Red blood cells (RBCs) and white blood cells (WBCs) were counted according to Feldman *et al*. (2000). Hemoglobin concentration and packed cells volume percentages were measured as described by Drew *et al*. (2004). Another 3 ml blood samples were collected into a clean

sterilized tube with heparin, non-coagulated blood samples were centrifuged at 4000 rpm for 15 min and the clear plasma was separated for the estimation of plasma triiodothyroxin (T_3), thyroxin (T_4) and growth hormones concentrations. Direct Radioimmunoassay (RIA) technique was used for the assessment of plasma triiodothyroxin (T_3) and thyroxin (T_4) concentrations as (ng/ml) using a kit according to the manufacturer´s instructions (DSLABS Webster Texas USA). The ratio between T3 and T4 (T3/T4) was also calculated. Plasma growth hormone concentration was measured by radioimmunoassay (RIA) (Gluckman *et al.,* 1989).

Table (2): Effect of growth hormone (GH) rabbit polymorphism individuals on live body weight (LBW), blood biochemical, hematological and jejunum histological characteristics of growing rabbits at 63 days of age (mean ± SE).

Traits	Genotype Individuals	
	AA	AB
Live body weight and blood biochemical parameters:		
LBW (g)	1678.7±29.3[b]	1833.4±21.2[a]
Triiodothyroxin (T_3) (ng/ml)	7.03±0.15[b]	7.86±0.11[a]
Thyroxin (T_4) (ng/ml)	33.20±0.63	33.25±0.45
T_3/T_4 (ratio)	4.72±0.13	4.23±0.09
Growth hormone (GH) (ng/ml)	30.38±0.46[b]	32.28±0.36[a]
Hematological parameters:		
Red blood cells (RBCs) (10^6/mm)	4.79±0.30[b]	5.10±0.07[a]
Hemoglobin (Hb) (g/dl)	13.35±0.64	14.02±0.13
Packed cells volume (PCV) (%)	40.13±1.35	40.45±0.27
White blood cells (WBCs) (10^3/mm)	7.92±0.29	8.20±0.06
Lymphocyte (%)	54.77±1.83[b]	59.72±0.37[a]
Jejunum histological characteristics:		
Intestinal Villi height (µm)	645.8±30.3[b]	789.2±27.4[a]
Crypt depth (µm)	139.4±8.3[b]	193.2±10.3[a]
Int. Musculosa thickness (µm)	107.6±10.0[b]	151.1±8.7[a]

[a and b] Means with different letters in the same row are significantly different at P≤ 0.05.

Molecular studies

Blood samples About 3 ml blood samples from each the previous slaughtered rabbit (12 ♀ and 12♂) per line was collected immediately after slaughter into a tube containing an anticoagulant disodium EDTA and stored at -20°C until needed for DNA extraction.

DNA isolation and PCR amplification

Genomic DNA was isolated from whole blood sample using a commercially available kit (GF-1 Blood DNA extraction kit-Vi Vantis, USA) and separated using 1% agarose gel in 0.5x TBE buffer to confirm DNA bands. GH sequence Primers for rabbit were designed according to GenBank, under accession number: Z38127 (Wallis and Wallis, 1995) to amplify a fragment of 345bp encompassing part of intron 1, exon 2 and part of intron 2 (forward: 5'CTAGCCTAGGGGAGGACTGG3'; reverse: 5'CTCATCCGACAGCATCTTCA-3'). PCR reaction was conducted according to

the manufacturer´s instructions of Master Mix (Promega). The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system.

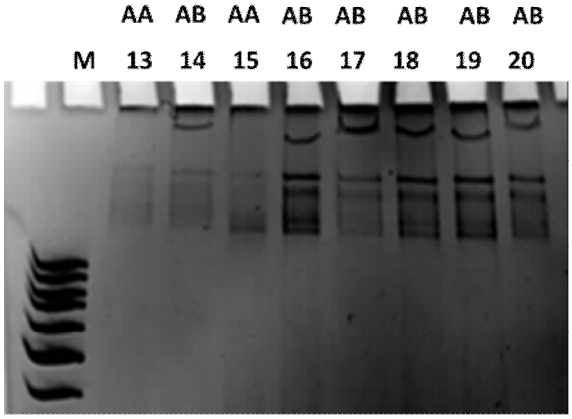

Fig.(1): Agarose gel electrophoresis of GH (345bp) DNA-SSCP in Alex line: where, lanes 13and 15→AA genotypes, 14, 16,17,18,19 and 20 →AB genotypes and M→ DNA marker (100 bp).

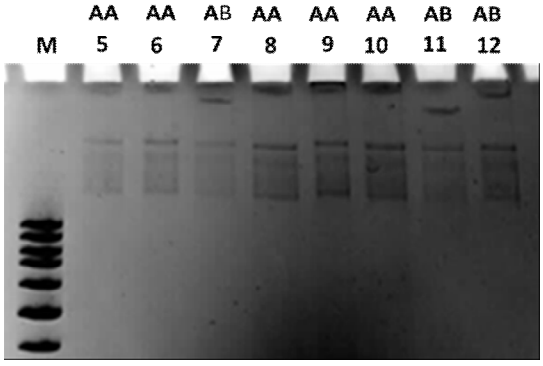

Fig.(2): Agarose gel electrophoresis of GH (345bp) DNA-SSCP in V line: where, lanes 5,6,8, 9 and 10 → AA genotypes, 7, 11 and 12 → AB genotypes and M→ DNA marker (100 bp).

Table (3): Effect of line and sex on live body weight (LBW), blood biochemical, hematological and jejunum histological characteristics of growing rabbit at 63 days of age (mean ± SE)

Traits		Effect Factors			
		Rabbit lines		Sex	
		V	Alex	Male	Female
LBW and blood biochemical parameters					
LBW	(g)	1734.9 ± 22.2	1795.9 ± 24.2	1775.8 ± 20.4	1755.0 ± 19.4
Triiodothyroxin (T_3)	(ng/ml)	7.10 ± 0.13^b	7.57 ± 0.14^a	7.72 ± 0.12^a	6.95 ± 0.18^b
Thyroxin (T_4)	(ng/ml)	32.64 ± 0.49	33.82 ± 0.44	34.29 ± 0.48^a	32.16 ± 0.36^b
T_3/T_4	(ratio)	4.66 ± 0.10	4.47 ± 0.13	4.47 ± 0.09	4.66 ± 0.11
Growth hormone (GH)	(ng/ml)	30.96 ± 0.42	31.62 ± 0.39	31.02 ± 0.48^a	29.56 ± 0.83^b
Hematological parameters					
RBCs	(10^6/mm)	4.78 ± 0.21^b	5.11 ± 0.21^a	5.35 ± 0.10^a	4.86 ± 0.11^b
Hb	(g/dl)	13.20 ± 0.33^b	14.17 ± 0.37^a	14.96 ± 0.18^a	13.06 ± 0.20^b
PCV	(%)	40.22 ± 0.68	40.37 ± 0.78	41.51 ± 0.37^a	39.32 ± 0.41^b
WBCs	(10^3/mm)	7.88 ± 0.15^b	8.24 ± 0.17^a	8.32 ± 0.08	8.09 ± 0.09
Lymphocyte	(%)	57.27± 0.93	57.22 ± 1.07	61.21 ± 0.55^a	57.84 ± 0.61^b
Jejunum histological characteristics					
Intestinal Villi height	(µm)	693.5 ± 35.8^b	741.0± 37.3^a	730.1 ± 43.3	705.0 ± 55.4
Crypt depth	(µm)	155.4 ± 11.4^b	169.2 ± 10.7^a	163.2 ± 7.5	161.9 ± 11.1
Int. musculosa thickness	(µm)	125.2 ± 12.2	134.1 ± 9.1	131.3 ± 9.1	127.4 ± 8.6

RBCs= Red blood cells; Hb= Hemoglobin; PCV= Packed cells volume; WBCs= White blood cells
$^{a \text{ and } b}$ Means with different letters in the same row are significantly different at P≤ 0.05

Genotyping by Single Stranded Conformation Polymorphism (SSCP)

Aliquots of 5µl PCR products were mixed with denaturating solution (98% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue and 10 mM EDTA) and incubated at 98C° for 10 min then chilled on ice rapidly. Denatured DNA was loaded on 10% PAGE gel (10X 10 CM) in 1X TBE buffer at 65V for 5 h. DNA bands were stained with ethidium bromide and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Histological examination

Twelve rabbits per line (6 ♀ and 6 ♂)) were sacrificed at the age of 63 days. Jejunum of small intestine Samples were obtained by the necropsy method (Jonathan *et al.*, 2016). The intestine was exposed and a sample of 0.5 cm from the middle part of jejunum was collected and placed it in 10 % neutral formalin solution for fixation. Slices with a 5-µm thickness were cut from each sample and stained with hematoxylin and eosin (Diapath S.R.L., Martinengo, Italy). The prepared samples were examined under a light microscope (Nikon Eclipse E600). Height of Intestinal Villus (µm) was measured from the top of the villus to the crypt transition. Crypt depth (µm) and intestinal musculosa thickness (µm) were also measured (Peter *et al.,* 2014). The crypt depth was defined as the invagination between two villi.

Statistical analysis

Allele and genotype frequencies were calculated in both populations according to Hardy-Weinberg law.

Polymorphism information content (PIC) and Gene heterozygosity (HE) were determined (Guo and Elston, 1999). The effects of different GH genotypes on the studied traits were analyzed using general linear model (GLM) procedure of the SAS software version (SAS, 2003) according to the following statistical model :

$Yik = \mu + GHi + eik$

Where: Yik = the observation of the trait studied, μ = the overall mean

GHi = the effect of genotype, i = (1 and 2), eik = the experimental random error.

While, the effects of line and sex on the studied traits were estimated as the following statistical model:

$Yijk = \mu + Li + Sj + eijk$

Where: $Yijk$ = the observation of the trait studied, μ = the overall mean

Li = the effect of line, i = (1 and 2),

Sj = the effect of sex, j = (1 and 2)

$eijk$ = the experimental random error.

Least significant range among different effect means was estimated (Duncan, 1955)

RESULTS AND DISCUSSION

Polymorphism analysis of rabbit GH gene

In the present study, SSCP-PCR was used to screen and identify nucleotide sequence polymorphism by changing in conformation of alleles within GH gene loci in the two lines of rabbit. Polymorphisms in the GH gene were identified within a part of intron1, exon2 and a part of intron2 of the gene .The present results in Table (1) and Figs. (1 and 2) indicated that this segment of GH gene displayed only two alleles A and B with AA and AB genotypes in the two lines. The A allele was the predominant with frequency of 0.83 and 0.54, respectively in V and Alex genotype individuals. AB genotype individual was the dominant genotype in Alex line because it showed high frequency (0.92) compared with AA genotype (0.08) while, it was shown to be lower (0.32) than AA homozygous individuals (0.72) in V-line (Table 1). Guo and Elston (1999) reported that heterozygosity (He) and polymorphic information content (PIC) are considered suitable parameters for estimating the genetic variation of populations, where high PIC reflects poor gene consistency, high genetic variability, and great genetic potentialities between individuals in a population. The results in Table (1) showed that GH locus was more informative ($0.25 < PIC < 0$) because its PIC and He were 0.373 and 0.497, respectively in Alex than V line, where the last one is represented as a moderate polymorphism (PIC=0.242) and (He=0.282) heterozygosity.

The effect of GH genotype individuals on live body weight (LBW) and blood biochemical parameters

A fraction of the genetic variability for the production traits which was observed between individuals in livestock might probably due to the variability within candidate genes (Rothschild and Soller, 1997). Currently, no research assessing the complete sequence of the rabbit GH gene on growth traits (Fontanesi *et al.*, 2012). The present study, showed statistically significant differences in the scores of LBW, T3 and growth hormone (GH) concentrations between AB and AA genotypes within a part of intron1, exon2 and a part of intron2 of the rabbit GH gene.

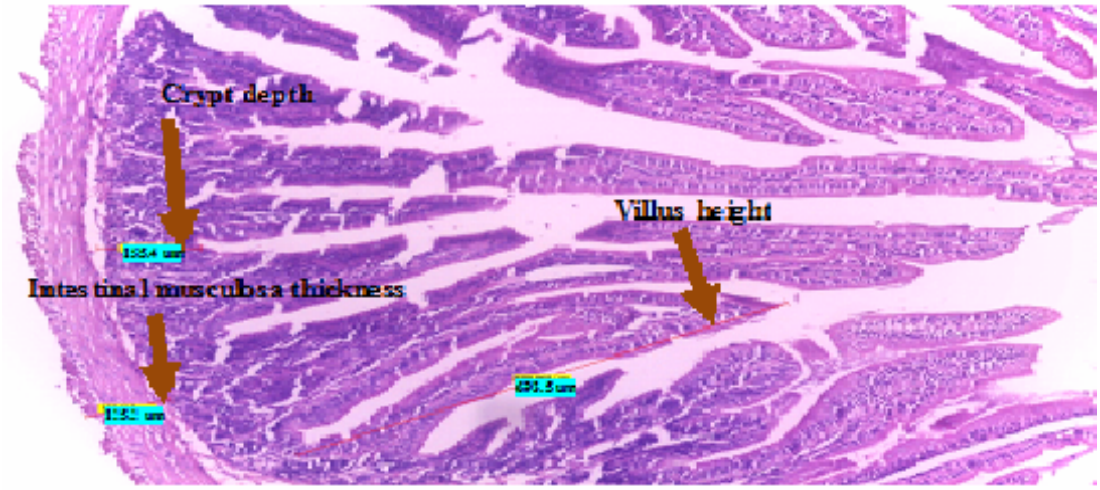

Fig. (3) : Photomicrograph in vertical section of Jejunum V line rabbits at 63 days of age, staining with hematoxylin and eosin (H & E) stains at 10 X mag. Arrows showed villi height, crypt depth and intestinal musculosa thickness.

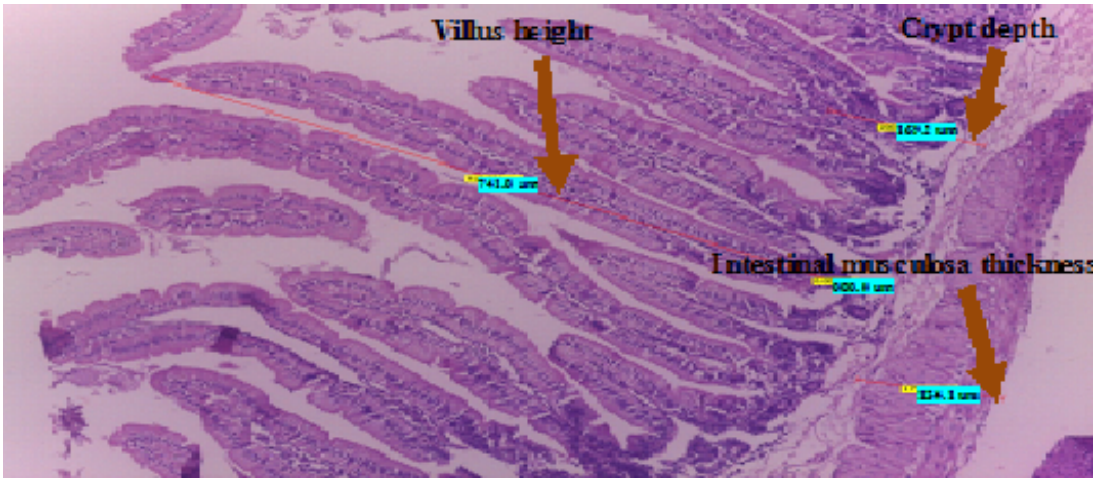

Fig. (4) : Photomicrograph in vertical section of Jejunum of Alex line rabbits at 63 days of age, staining with hematoxylin and eosin (H & E) stains at 10 X mag. Arrows showed increasing in compared with fig.3 in villi height, crypt depth and intestinal musculosa thickness.

AB genotype recorded higher significant effect on LBW at 63-day of age, T3 and growth hormone values than AA genotype at P ≤ 0.05. The percentages of that increase of AB compared with AA genotypes were 9.2, 11.8 and 6.3 % in LBW, T3 and the concentration of GH, respectively as shown in Table 2. In a previous investigation reported by Abd-El-Ghany (2007) who found that heterozygote genotypes associated with the heavy weight at different ages during the growth period in rabbits and this increase in the weight was high significant at 8 weeks of age. Jia *et al.* (2014) studied the correlation between polymorphisms in the 5' regulatory region, exon 4, and 3' untranslated region (UTR) of the sheep GH gene and sheep growth traits. He found that there were statistically significant differences in the scores of weight, length, and heart girth within the 5' regulatory region; weight, length, wither height, and heart girth within exon 4; and weight, length, wither height, and heart girth within the 3' UTR among the different genotypes in Tibetan sheep . AC heterozygote genotype showed higher scores than those of genotypes AA and AB (P<0.05) within3' UTR in Poll Dorset sheep. Zhang *et al.* (2012) stated that the polymorphisms of GH gene were always associated with production traits which make it as a candidate gene to enhance the productivity of livestock and fowl.

Blood hematological characteristics

Blood hematological indicators provide valuable information on the health status of animals. Many researchers observed the effect of genetic makeup on hematological and biochemical status of rabbits (Cazabon *et al.,* 2000).The present results showed that all values of hematological indicators fall within the normal range and this reflects the vital physiological relationship of haemoglobin with transporting gases to and from the tissues of the body was normal (Njidda *et al*., 2006). Also, no significant differences were observed between AA and AB of GH genotype individuals in hematological indicators, except RBCs and Lymphocyte parameters (Table 2). Our results corresponded with Chineke *et al*. (2006) who reported that the effect of genotype on PCV, WBC and HBC values were identical in all genotype individuals, pointing similar cellular haemoglobin content in the obtained blood samples. The current results referred to the significant differences between AB and AA genotypes in RBCs and Lymphocytes percent. The percentages of that increase of AB compared with AA genotypes were 6.5 and 9.0% in RBCs and Lymphocytes, respectively as shown in Table (2). This increment in RBCs and Lymphocytes percentages may be attributed to the physiological and nutritional status of these animals (Esonu *et al*., 2001). Also, these variations in some blood components may be due to the genetic variations between genotype individuals, which play an important part in the productive and reproductive traits (Abdel-Azeem *et al.*, 2007).

Effect of line and sex on live body weight and hemato -biochemical parameters

The results shown in Table (3) revealed that serum T3, T4 and GH concentrations were significantly affected by sex since; the males had significantly higher value in these traits than females by an 11.1, 6.6 and 4.9% increase, respectively. On the contrary, lines didn't show any significant differences in LBW, T4, T3/T4 ratio and GH concentrations except in T3 as Alex line showed higher significant level in this trait than those in V-line by an increase of 6.65%. In a previous

study, suggested that thyroid hormone stimulates the production of growth factors which will influence body growth (Cabello and Wrutniak,1989). This is consistent with our results that the synthetic paternal line (Alex) is characterized by high rate of growth than the other synthetic maternal line (V line). El-Raffa (2010) suggested that the criteria of Alex line selection is daily weight gain from weaning to slaughter age that was used as a selection goal. The hematological parameters including Hb, RBCs, PCV and Lymph values were also affected by sex except WBCs. The males had significantly higher values than those recorded in the females. This increase in males reached to 110.0, 114.5, 105.6 and 105.8% in RBCs, Hb, PCV and Lymph parameters, respectively as shown in Table 3. Our results are in agreement with Khalil *et al.* (2014) who found that males always showed increment in hematological parameters and this increment may be related to sexual hormones. Also, Ozegbe (2001) interpreted the reduction in the erythrocyte count and hemoglobin values in the female compared with males may be related to the physiological anemia occurring due to hemo-dilution. Hb, RBCs and WBCs values were affected by lines since; Alex line showed higher significant value ($P \leq 0.05$) than V line in the previous parameters as shown in Table 3. The percentages of that increase of Alex line compared with V line rabbits were 6.9, 7.3 and 4.6% in RBCs, Hb and WBCs, respectively. This increment may be due to the fact that Alex line was established and developed at Egyptian conditions so it is considered as a native line that have higher adaptation than (imported line) V line (Khalil *et al.,* 2014).

Histological study

Histological study is considered as a good indicator for good health status and increase in body weight of animals because the changes in gut morphology referred to increase surface area for nutrient absorption consequently, releasing of digestive enzymes (Jungbauer *et al*., 2011). The present results indicated that Jejunum villi height, crypt depth and intestinal musculosa thickness of the growing rabbits were significantly ($P \leq 0.05$) increased with AB Genotype individuals. The percentages that increase of AB compared with AA Genotype individuals were 22.2, 38.6 and 40.4% in Jejunum villi height, crypt depth and intestinal musculosa thickness, respectively as shown in Table (2). The histological results were found to be associate with the above results in the current study where, AB genotype was always superior in the most studied traits. The present results are supported by several histological studies as Sims *et al.* (2004) who noticed that the lengthened villi are associated with superior gut health as well as improved nutrient absorption consequently, increasing in body weight .Also, The villus growth correlates well with the various stages of growth, increased body weight with the increase in relative weight of the intestinal tract (Bi et *al.*, 1997). The crypt cells can be regarded as "villi factory" and a large crypt cell indicates fast tissue turnover, which increases the nutrient requirement for maintenance of the gut wall (Jungbauer *et al.*, 2011). The effect of line and sex on jejunum of Intestinal Villi height, Crypt depth and Intestinal musculosa thickness were also studied in the present investigation. The intestinal Villi height and Crypt depth recorded a significant increase in Alex line at $P \leq 0.05$ compared with V line as shown in Fig. (3, 4) and Table (3). The increase percent of intestinal Villi height and Crypt depth in Alex line were 106.8 and 108.9%, respectively while; the intestinal mucosal thickness was not affected by line as shown in Table (3). Our results may reflect the increased of live body weight of Alex line compared with V line

rabbits, however the criterion of selection for Alex line (Paternal line) was to gain daily weight from weaning to slaughter age (El-Raffa, 2010), whereas number weaning was a criterion of selection in V-line rabbits (Baselga, 2002). On the other hand, there is no significant difference between males and females in these histological traits in both lines (Table 3). The current study concluded that AB genotype was always superior in the most studied traits in both lines that could be fixed as a favorable genotype in rabbits. Alex line exhibited higher score in some studied traits than V line. A histological study was important for supporting the above results. Screening of rabbit GH gene by PCR-SSCP may apply this gene as candidate gene for further applications in marker-assisted selection in rabbit breeding program.

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الملخص العربي

تحليل تعدد الاشكال لجين هرمون النمو للأرانب وتقييم تأثيرة على وزن الجسم وعلى صفات الدم الهيماتو – بيوكيميائية

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يلعب هرمون النمو دور هام فى النمو والتمثيل الغذائي في الثدييات. وقد درس تعدد الأشكال الوراثية لهذا الجين وارتباطة ببعض الصفات الانتاجية فى العديد من الحيوانات المزرعية. الهدف من هذة الدراسة هو دراسة تعدد الأشكال الوراثية فى المنطقة جزء من إنترون ١، إيكسون ٢ وجزء من إنترون ٢ من هذا الجين فى الارانب وتقييم تأثيره على وزن الجسم الحى وبعض صفات الدم الهيماتو- البيوكيميائية. وبالتالى، تم اختيار أربعة وعشرين أرنب (١٢ ♀ و ١٢ ♂) عشوائياً من خطوط الأسكندرية والـ V لسحب عينات دم لإستخلاص الـ DNA وتحليل صفات الدم الهيماتو-بيوكيمائية. استخدام تكنيك تفاعل البلمرة المتسلسل فى وجود بادئات متخصصة لتكبيرشظية مقدارها bp ٣٤٥ من جين هرمون النمو. درست تعدد الاشكال الوراثية داخل كل سلالة من خلال تكنيك SSCP. أظهرت النتائج وجود اليليين هما A, B وتركيبن وراثين AA, AB فى كلا من العشيرتين التى تحت الدراسة وكان التركيب الوراثى الخليط AB هو السائد فى خط الأسكندرية بينما كان التركيب الوراثى المتماثل AA هو السائد فى الخط V. وصفة وزن الجسم بالمقارنة بالتراكيب المتماثلة كانت أكثر تأثيراً فى خط أسكندرية عن خط V. أظهرت التراكيب الوراثية الخليطة AB اختلافات معنوية فى معظم الصفات المدروسة. خط أسكندرية والذكور تفوق بفروقات معنوية فى معظم الصفات المدروسة. النتائج المذكورة سابقاً أرتبطت مع الفحص الهستولوجى لطول الخملات وعمق Crypt والغشاء العضلى بالأمعاء الدقيقة حيث زادت معنوياً مع التركيب الوراثى الخليط AB وخط أسكندرية. لذا دراسة جين هرمون النمو للارانب بواسطة تكنيك الPCR-SSCP لتقييم تعدد الاشكال الوراثية وعلاقتها مع بعض الصفات الأقتصادية قد تكون مفيدة لتطبيق هذا الجين كعلامة مساعدة للأنتخاب في برنامج تربية الأرانب.