SINGLE - STRAND CONFORMATION POLYMORPHISM OF *MYOSTATIN* GENE LINKED TO SLAUGHTER TRAITS AND SOME BLOOD CONSTITUENTS IN RABBITS

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The *myostatin* (*MSTN*) gene is a member of the transforming growth factor- β superfamily .It is called growth differentiation factor 8 (GDF8). *MSTN* gene codes for a growth factor that actively represses skeletal muscle growth by inhibiting cell cycle progression which determines the maxim growth of body mass for every species (Fontanesi *et al*., 2011). The sequence and function of *MSTN* gene appears to be highly conserved among vertebrates through evolution (Scheuermann *et al*., 2004). Many studies was carried to induce mutation in this gene in which disrupting *MSTN* gene function and causes an increased in number of muscle fibers followed by muscle cell hypertrophy and suppression of body fat accumulation as the mice with mutated *MSTN* gene generated mice showed a 2 to 3-fold increase in muscle mass compared with wild-type mice (McPherron *et al*., 1997). In dogs, a disrupting mutation in exon 3 of this gene resulted in a double-muscled phenotype linked to enhanced racing performance (Mosher *et al*., 2007). Several mutations of *MSTN* gene impaired expression of *MSTN* gene function and caused double muscle phenotypes, muscle hypertrophy, birthing difficulties, increased growth rate and increased feed conversion efficiency in cattle (Marchitelli *et al*., 2003). Also, the role of *MSTN* gene during myogenesis of chickens was expected to be similar to that observed in mammals (Scheuermann *et al.*, 2004).

The rabbit (*Oryctolagus cuniculus*) is an animal of high economic value where, its meat has high nutritional quality. It contains a high amount of protein and polyunsaturated fatty acids in addition to low amounts of fat and Cholesterol (Das and Bujarbarua, 2005) .The rabbit industry in Egypt has shown interest to increase rabbit production in the last decades which may be one of the solutions to overcome the shortage of red meat problem.

The sequence of rabbit *MSTN* gene comprises three exons and two introns as in the other species (Ensembl Gene ID:

NSOCUG00000012663, http://www.ensembl.org/Oryctolagus_cuniculus/index.html). Few studies have been carried out so far to investigate its variability or the effects of MSTN polymorphisms on rabbit production traits. Fontanesi *et al.* (2008) detected a SNP which is a C-T transition in position 34 in a part of intron 2 causing a polymorphic site in rabbit *MSTN* gene. Also, Kurkute *et al.* (2011) studied three *MSTN* exonic regions in three different rabbit breeds and found one variation (A>G) in exon 1 in the Soviet chinchilla and one variation (G>A) in exon 3 in the White giant breed that can be used as one of the candidate markers for meat production trait. Peng *et al.* (2013) stated that a mutation in 5'regulatory region of *MSTN* gene could lead to change of the gene expression and thereby influence growth and carcass traits of animal. Abdel-Kafy *et al*. (2016) studied the polymorphic site in exon 2, intron 2 and 3'-untranslated regions in rabbit *MSTN* gene and found that GG genotype at the C.194A>G SNP in 3'-untranslated region showed significant effect on most carcass traits, in addition a significant association was shown between SNP (c.747+34c > T) in intron 2 of the gene and body weight. Ibrahim and Hickford (2015) used Polymerase Chain Reaction Single-Strand Conformation Polymorphism (PCR-SSCP) analysis to identify the allelic and genotypic polymorphisms in intron 1 of *MSTN* gene in males of New Zealand Romy lambs. They found that there were associations of the variations in intron 1 of *MSTN* gene with carcass traits. Therefore, the present investigation focused on studying the polymorphism of *MSTN* gene within a part of intron 2, the coding sequence of exon 3 and a part of the 3'untranslated region of rabbit *MSTN* gene using PCR-SSCP method and studying its impact on carcass traits and some blood constituents as plasma total protein, Albumin, Globulin, serum Ca and IP in each of V and Alex rabbit lines. These segments of the gene were chosen on the basis that they represent intron, exon and UTR (different parts) of *MSTN* gene. Also, the effects of sex and line on the above traits were investigated to provide information basis for selection of a favorable line that can be useful in rabbit breeding program.

MATERIALS AND METHODS

Animal experiment and management

Animal experiment was conducted during the season of 2014/2015 at rabbitry of the Poultry Research Center of the Poultry Production Department, Faculty of Agriculture, Alexandria University, Egypt. Two lines were used in the present study, the first was V line that is considered a synthetic maternal line, originated in Spain and was selected for its high productivity and litter size at weaning (Baselga, 2002).The second was Alexandria (Alex) line which was established in Egypt, selected for increasing of daily weight from weaning to slaughter age and taken as a new synthetic paternal rabbit (El-Raffa, 2010).

A total of 410 unsexed weaned individual rabbits from two lines including

210 V line and 200 Alex rabbit line were selected at four weeks of age with an average initial weight of 540±20 g. These individuals were taken from dams characterized by high production in number of bunnies at birth, high number of weaned rabbits per doe and high weight of bunnies. These weaned individuals were individually ear-numbered, housed in progeny cages after dividing to groups and reared to 9 weeks of age (Slaughter age). Animals were given ad libitum access to commercial pelleted diet containing 17.85% crude protein, 11.89% crude fiber, 2.75% fat and 2556 kcal/kg diet. They were kept under high standards of hygiene and good management.

Blood samples and DNA extraction

Twenty four rabbits (12♀ and 12♂) at age of 9 weeks (Slaughter age) were randomly chosen from each line for collecting of blood samples. About 1 ml blood sample was drawn from the marginal ear vein of each rabbit under vacuum, collected into a coated tube with disodium EDTA and stored at -20°C for DNA extraction. Total genomic DNA was isolated from the whole blood sample using DNA purification kit according to the manufacturer´s instructions (GF-1 –Vivants, USA) to be used as a source for template DNA.

PCR and genotyping of MSTN gene by SSCP

A primer pair of *MSTN* gene previously reported by (Fontanesi *et al.*, 2008) as (forward: 5'AAAGGTATTCCAAGCAAAATGA3'; reverse 5'GGGGAAGACCTTCCATGTTT3') was used to amplify a fragment of 523 bp in the *MSTN* gene including a part of intron 2, the coding sequence of exon3 and a part of the 3'untranslated region. PCR reaction was conducted in a volume of 25 µl containing 25 ng of genomic DNA of each sample, 25 pmol of primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/µl Taq DNA polymerase (Promega, Germany). Thermal cycling (MyGene Series Peltier Thermal Cycler) was carried out by initial denaturation at 94°C for 5 min, followed by 35 cycles each at 94°C for 45 sec, annealing temperature at 58°C for 40 sec, polymerization temperature at 72°C for 45 sec and final extension at 72°C for 10 min, then the samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator. The amplified former region of all samples was screened by SSCP method where, 5 µl of sample buffer (denaturing solution) containing 98% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue and 10 mM EDTA, pH 8.0 which was mixed with aliquots of 1 µl PCR product and denatured at 98°C for 10 min then chilled on ice bath rapidly for 10 min to retain the denatured state. Denatured DNA was loaded on 10% PAGE gel (10X 10 CM) in 1X TBE buffer at 65V for 5 h for electrophoresis of the products. DNA bands were stained with ethidium bromide and photographed by Gel Documentation system (Alpha Imager M1220, Canada).

Slaughter traits

Slaughter traits were measured in the above individuals for each line where, animals (12♀ and 12♂) were taken at the end of the experiment (9 weeks), fasted for 12 hours before slaughtering according to (Blasco and Ouhayoun, 1996) then the animals were weighed individually and slaughtered to complete bleeding (Cheeke, 1987). After bleeding, rabbits were weighed skinned and weighed again after skinning to calculate the pelt weight. After slaughtering and skinning, the carcass was eviscerated and dressing percentage was estimated for each animal by dividing the relative weight of hot eviscerated carcass including giblets, abdominal fat and head by the weight of live body weight as described by (Steven *et al.*, 1981). While, non-carcass fat included relative weight of carcass, giblets of liver, heart, pancreas, thyroid and abdominal fat were separately weighed and each of them was proportioned to the live pre-slaughtering weight. Slaughter length was also measured to the nearest centimeter.

Biochemical analysis

The same above individual rabbit (12♀ and 12♂) at the same age (9 weeks) from each line with a total of 48 animals from the two lines were also taken at 08:00-09:00 am. before slaughtering to collect blood samples for Biochemical analysis. A blood sample of about 3 ml was pulled from the marginal ear vein of each animal under vacuum and put it into a clean sterilized tube that coated with heparin (non-coagulated blood) for estimation of total protein, albumin, globulin and glucose. Another blood sample was collected into a clean sterilized tube (coagulated blood) for measuring calcium (Ca) and Inorganic phosphorus (IP) in serum. Coagulated and Non-coagulated blood samples were centrifuged at 4000 rpm for 15 min then the clear serum and plasma were separated and stored in a deep freezer at -20°C until usage. Blood constituents including plasma total proteins and albumin, were measured using suitable commercial kits according to guidelines (Bogin and Keller, 1987). The level of Globulin was estimated by subtracting the value of albumin from the corresponding value of total protein. Albumin to Globulin ratio (A/G) was also calculated. Plasma glucose concentration was measured using colorimetric method as described by (Trinder, 1969). Serum calcium (Ca) and Inorganic phosphorus (IP) were measured by a colorimetric method using commercial kits of Sclavo Diagnostics Company (Kite Italia S.P.A.) as described by (Hawk's, 1965).

Statistical analysis

PCR-SSCP data were analyzed by calculating allelic and genotypic frequencies. The heterozygosity (He) of a locus and polymorphic information content (PIC) of allele were calculated as described by Liu (1998) using the following formula.

$$He = 1 - \sum_{i=1}^{l} P_i^2$$

Where, P_i is the frequency of the *i*th allele among a total of *l* alleles.

$$PIC = 1 - \sum_{i=1}^{1} P_i^2 - \sum_{i=1}^{L-1} \sum_{j=i+1}^{L} 2\, P_i^2\, P_j^2$$

Where, P_i and Pj are the population frequencies of the *i*th and *j*th alleles, L is the number of alleles from a certain locus

The effect of *MSTN* genotype polymorphisms on different traits were estimated using general linear model (GLM) procedure of the SAS software (SAS, 2003) as the following statistical model:

$$Y_{ik} = \mu + MSTN_i + e_{ik}$$

where: Y_{ik} = observation of the studied trait, μ = overall mean, $MSTN_i$ = effect of *MSTN* genotype, i = (1 and 2) e_{ik} = experimental random error.

The effects of line and sex on the studied traits were studied using the following statistical model:

$$Y_{ijk} = \mu + L_i + S_j + e_{ijk}$$

where: Y_{ijk} = the observation of the studied trait, μ = overall mean, Li = the effect of line, i = (1 and 2), S_j = effect of sex, j = (1 and 2), e_{ijk} = experimental random error.

Least significant range among different effect means was estimated according to (Duncan 1955).

RESULTS AND DISCUSSION

Allelic and genotypic frequencies of MSTN gene in rabbit lines

The result of PCR-SSCP analysis showed that the 523 bp *MSTN* gene segment which was located in a part of intron 2, the coding sequence of exon3 and a part of the 3'untranslated region were polymorphic in Alex and V lines. In this locus, two SSCP patterns were detected and identified as AA and AB genotypes in V-line while, only one SSCP pattern was seen in all samples of Alex line which was characterized as AB genotype as shown in Figs (1 and 2). Two alleles namely, A and B alleles were derived from the two identified genotypes in the two lines. AB individual was the predominant genotype in V line because it showed high frequency (0.92) compared with AA genotype (0.08) while, the population of Alex line was shown to be heterozygous (AB) with a genotypic frequency being 1.00. The allelic frequency was 0.54 and 0.50 for A allele and 0.46 and 0.50 for *B* allele in V and Alex populations, respectively as shown in Table (1). The analysis results of PIC and He in the studied region of the gene revealed that values of PIC and He were 0.497 and 0.373 in V population while, it was 0.500 and 0.375 for PIC and He in Alex population, respectively, as shown in Table (1). Nei and Kumar (2000) stated that an allele is polymorphic if its frequency was equal or less than 0.99. Renee *et al.* (2009) and Liu (1998) defined the heterozygosity (He) of a locus as the probability in which an individual is heterozygous for this locus in the population while, PIC provides an estimate of the discriminating power of the marker and thereby it refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency. Also, Carl *et al.* (1992) report-

ed that a gene or marker with only two alleles has a maximum PIC of 0.375. Based on the previous studies, the region of *MSTN* gene locus in the present study was considered to be highly informative in the two populations ($0.25 < PIC < 0$) but it was more informative in Alex than V line and this because the population of Alex line under study included heterozygous individuals.

Effect of MSTN genotype on slaughter traits

Rothschild and Soller (1997) mentioned that DNA polymorphism associated with economic traits can be identified by studying candidate genes that are directly or indirectly correlated to the physiological mechanisms influencing important traits for selection. Fontanesi *et al*. (2008) reported that the variability within genes coding for protein products might play an important role in explaining a fraction of genetic variability for the productive trait itself. *MSTN* gene polymorphisms and their association with different performance traits were successfully determined in domestic rabbits (Qiao, 2010; Abdel-Kafy *et al.,* 2016). In the present study, the association analysis between genotypes of *MSTN* gene polymorphisms and slaughter traits in 48 rabbit individuals indicated an increase in Carcass (%), dressing (%), liver (%) and carcass length (cm) in AB *MSTN* genotype achieving significantly ($P \leq 0.05$) higher score for these traits than AA genotype individuals by 7.7%, 2.2%, 11.1% and 3.3%, respectively. On the other hand, there were no significant differences between AA and AB *MSTN* Genotype individuals in heart (%), pancreas (%), thyroid (%) and abdominal fat (%) traits as shown in Table (2). Sternstein *et al*. (2014) studied the polymorphisms of the *MSTN* gene in a cross between Giant Grey (GG) and New Zealand White (NZW) rabbits to evaluate its effect on carcass composition and meat quality traits. They found a significant association of MSTN SNP c. 373+234G>A ($P < 0.05$) which is located in intron1 with nine carcass composition traits including skeletal muscle and bone weights. Qiao *et al. (*2014) analyzed the polymorphisms of all the three exon regions and a part of the 5'-regulatory region of the *MSTN* gene by sequencing and single-strand conformation polymorphism techniques in nine pure lines of rabbit and their hybrid combinations for studying its impact on meat quality. The study revealed that the body weight at 70 and 84 days of age and chest circumference traits were significantly higher (($P < 0.05$) in AA genotype than AB and BB genotypes. Single nucleotide mutation (T→C) on the 476 locus of the 5'-regulatory region of the gene affected the cooked muscle rate, drip loss, and some carcass traits. Ibrahim and Hickford (2015) studied the polymorphisms in intron 1 of *MSTN* gene in New Zealand Romy sheep using SSCP-PCR and found that MSTN genotypes significantly affected slaughtering weight and total yield; also it highly affected dressing%, leg yield% and shoulder yield%.

Effect of MSTN genotype on blood constituent's parameters

Plasma total proteins of animal consist of albumin and globulin. It is known that the liver is the site of albumin synthesis and the concentration of albumin always reflected the animal ability to synthesize and store protein subsequently reflecting the hepatic function of animal. Meanwhile, the ratio between albumin and Globulin is a good indicator for increasing the immunoglobulin in plasma proteins of mammals (Ashour *et al*., 2004). The results of blood constituents in the present study revealed that all parameters i.e. plasma total protein, Albumin, Globulin; A/G ratio and serum Ca and IP were not affected by *MSTN* genotypes except for the level of glucose. Where, AA Genotype recorded significant higher effect in glucose level than AB genotype by an increase of 5.7% as shown in Table (2). Martinec *et al.* (2012) stated that some serum biochemical parameters were not affected by genotype. On the other side, Isaac *et al.* (2013) reported that some variations in Biochemical parameters could be affected by the rabbit genotype and these variables are genetically resistant to certain diseases and environmental conditions.

Effects of line and sex on slaughter traits and blood constituent's parameters

Slaughter traits including Carcass%, Dressing%, liver%, Heart%, Pancreas%, Thyroid% and Carcass length (cm) were not significantly affected neither by the line nor sex except for abdominal fat%, which was the only trait that had significant difference ($P \leq 0.05$) in Alex line (line effect). It showed significantly ($P \leq 0.05$) higher difference by an increase of 10.5% than V line as shown in Table (3). This increase in abdominal fat% may be due to its physiological and nutritional status of this native parental line (Alex line) that is adapted to Egyptian conditions (Khalil *et al.*, 2014). In addition, the criterion for Alex line selection is to gain daily weight from weaning to slaughter age (El-Raffa, 2010). The result of blood constituent's parameters showed that only plasma total protein, Globulin values and A/G ratio were affected by sex where, the males realized significantly ($P \leq 0.05$) higher score by an increase of 8.6 and 16.4% in total protein and Globulin, respectively than females for these traits. While, A/G ratio was significantly higher ($P \leq 0.05$) in females by 12.8% increase than males rabbits (Table 3). Dede *et al.* (2014) found that serum proteins fractions varied in quantity and quality according to physiological conditions of animal such as age, gender and pregnancy. Also, some environmental conditions such as nutrition; some diseases and temperature. Durai *et al*. (2012) reported that the ratio between albumin and globulin can be considered as a reference value and may serve as a guide to assess the state of health in the monitored animal. The rest of the biochemical values (Albumin, Glucose, Ca and P) under study failed to show any significant difference between males and females as shown in Table (3). Marchal *et al.* (2012) found that no significant differences were observed in albumin, glucose,

Ca and Phosphorus levels between males and females in mouse lemurs. Abdel Azeem et *al.* (2010) stated that Sex in rabbits had no significant effect on albumin and glucose levels. All blood constituents (Total protein, Albumin, Globulin, Glucose, Ca and P) were not affected by lines in the current study. Also, slaughter traits and blood constituent's parameters were not significantly affected by the interaction between line and sex (Table 3).

Finally, the obtained experimental results confirmed that PCR-SSCP is an appropriate tool to detect the variability of *MSTN* gene as a marker gene affecting important traits in rabbits. The identified polymorphisms in this region of *MSTN* gene were important for their allele and genotypic frequencies distribution in the two populations of rabbit. it is possible to select the AB genotype, which has always been superior in most slaughter traits. Our study pointed to some importance in blood constituent's parameters as total protein, globulin and the ratio between albumin and globulin which are affected by gender and can be used to assess the health as well as the physiological status of farm animals. Finally, additional regions for *MSTN* gene using modern techniques should be studied to assess the effect of variation in other regions of this gene that might provide more information about *MSTN* gene as DNA markers in the future.

SUMMARY

Myostatin (*MSTN*) gene expressed mainly in muscles of animals and human. It acts as a negative regulator of skeletal muscle development that limits skeletal muscle mass. Several mutations have been identified in the *MSTN* gene which naturally occurred in this gene leading to increase in skeletal muscle mass consequently better growth performance for animal. It has been considered as a candidate gene for meat selection programs in domestic animals. Therefore, this study aimed to use Polymerase Chain Reaction Single-Strand Conformation Polymorphism (PCR-SSCP) method to explore genetic polymorphisms of the part of intron 2, the coding sequence of exon 3 and a part of the 3' untranslated region of *MSTN* gene in rabbits. Also the study evaluated genetic polymorphism of the *MSTN* gene on slaughter traits and some blood constituents as plasma total protein, Albumin, Globulin, serum Ca and IP in addition to the effect of line and sex on the above traits was also studied in the present investigation. So, twenty four rabbits (12♀ and 12♂) at 9 weeks of age (Slaughter age) from each V and Alex lines were selected for genotyping this segment of rabbit *MSTN* gene. The analysis of PCR-SSCP revealed two SSCP patterns i.e., AB and AA in V population with a genotypic frequency of 0.92 and 0.08, respectively. While, Alex population showed one type of pattern i.e. AB with a frequency of 1.00 in the *MSTN* gene. The two SSCP genotypes had two alleles i.e. A and B alleles. The frequency of A allele was 0.54, 0.50, while the B allele showed a frequency of 0.46 and 0.50, respectively in V and Alex populations. *MSTN* locus was highly informative and showed high heterozygosity

in both lines. AB genotype always recorded higher score in carcass%, dressing%, liver % and carcass length than AA genotype. Plasma glucose concentration was affected by MSTN polymorphisms that revealed significant differences with AA compared with AB genotype. Alex line had significant influence on abdominal fat percentage. Also, sex had significant effect on total protein and globulin (g/d) as males showed significant increase in these traits than female's rabbits.

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Table (1): Genotypic and allelic frequencies for rabbit *MSTN-SSCP* and its genetic characteristics in V and Alex lines.

Primer characteristics	Lines	Genotypic frequencies		Allelic frequencies		Genetic Characteristics	
		AA	AB	A	B	PIC	He
MSTN (523 bp)	V	0.08	0.92	0.54	0.46	0.497	0.373
	Alex	0.00	1.00	0.50	0.50	0.500	0.375

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

Table (2): Effect of *MSTN* genotypes on slaughter traits and blood constituents' parameters of rabbits at 63 days of age (mean±SE).

Traits	Genotypes	
	AA	AB
Slaughter traits		
Carcass %	56.31±2.62[b]	60.62±3.23[a]
Dressing %	61.80±3.52[b]	63.15±2.22[a]
Liver %	3.06 ± 0.19[b]	3.40 ± 0.11[a]
Heart %	0.374±0.056	0.385±0.023
Pancreas %	0.333±0.062	0.354±0.017
Thyroid %	0.017±0.003	0.023±0.004
Abdominal fat %	1.011±0.034	0.920±0.052
Carcass length cm	32.10 ±2.86[b]	33.16 ±1.74[a]
Blood constituent's parameters		
Total protein g/dl	6.49±0.15	6.75±0.11
Albumin g/dl	3.63±0.14	3.82±0.10
Globulin g/dl	2.86±0.17	2.93±0.12
A/G ratio	1.27±0.11	1.30±0.10
Glucose mmol/L	7.24±0.10[a]	6.85±0.07[b]
Ca mmol/L	2.89±0.08	2.90±0.06
P mmol/L	2.89±0.08	1.25±0.03

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

Table (3): Effects of line, sex and their interaction on slaughter traits and blood constituents' parameters of rabbits at 63 days (mean±SE).

Traits	Rabbit Lines		Sex		Interaction between Lines and Sex			
					V line x sex		Alex line x sex	
	V	Alex	Male (M)	Female (F)	V × M	V × F	Alex × M	Alex × F
Slaughter traits								
Carcass %	57.78 ±1.85	59.15±2.03	58.62±2.78	58.33±3.56	58.20±4.76	58.06±4.87	58.89±2.95	58.74±3.65
Dressing %	62.38 ±3.18	63.71±2.94	63.26±1.73	62.84±3.26	62.82±5.22	62.61±3.65	63.49±4.48	63.28±3.39
Liver %	3.20±0.15	3.26 ± 0.19	3.39 ± 0.20	3.07 ± 0.23	3.30 ±0.36	3.14 ±0.55	3.32±0.41	3.17 ± 0.41
Heart %	0.38±0.07	0.38 ± 0.03	0.39 ± 0.02	0.37 ± 0.02	0.38 ±0.03	0.37 ±0.06	0.39±0.04	0.38 ±0.02
Pancreas %	0.34±0.07	0.35 ± 0.03	0.34 ± 0.01	0.35 ± 0.01	0.34 ±0.06	0.35 ±0.08	0.34±0.06	0.35 ±0.03
Thyroid %	0.017±0.01	0.022± 0.01	0.020±0.01	0.022±0.02	0.019±0.01	0.020±0.01	0.021±0.01	0.022±0.01
Abd. Fat %	0.92±0.03^{b}	1.01 ±0.03^{a}	0.86 ±0.04	1.07 ± 0.02	0.89 ±0.04	0.99 ±0.03	0.94±0.04	1.04 ±0.04
S. length cm	32.14±2.22	32.13±3.56	32.25±2.39	32.02±1.22	32.20±2.19	32.08± 2.82	32.19±2.95	2.08±2.09
Blood constituent parameters								
T. protein g/dl	6.68±0.20	6.63±0.23	6.93±0.23^{A}	6.38±0.20^{B}	6.81±0.38	6.53±0.37	6.75±0.18	6.51±0.28
Albumin g/dl	3.76±0.21	3.62±0.18	3.74±0.21	3.63±0.18	3.75±0.16	3.69±0.27	3.68±0.23	3.62±0.25
Globuling/dl	2.92±0.22	3.02±0.25	3.19±0.25^{A}	2.74±0.22^{B}	3.06±0.15	2.83±0.28	3.11±0.23	2.88±0.34
A/G ratio	1.29±0.08	1.21±0.12	1.17±0.11^{B}	1.32±0.09^{A}	1.23±0.05	1.30±0.09	1.20±0.09	1.25±0.12
Glucose mmol/L	7.08±0.15	7.04±0.17	7.37±0.17	6.75±0.15	7.23±0.21	6.92±0.20	7.20±0.16	6.90±0.17
Ca mmol/L	2.88±0.10	2.86±0.12	2.91±0.12	2.83±0.11	2.89±0.14	2.86±0.18	2.88±0.13	2.85±0.14
P mmol/L	1.25±0.05	1.23±0.06	1.24±0.06	1.24±0.05	1.25±0.07	1.24±0.02	1.23±0.08	1.23±0.10

V = V line; Alex = Alexandria line; Abd. Fat = Abdominal Fat; S.lenght = Slaughter length ; A= Albumin; G = Globulin

$^{a, b...}$ Means of rabbit lines with different letters in the same row are significantly different at P≤ 0.05

$^{A, B..}$ Means of sex with different letters in the same row are significantly different at P≤ 0.05

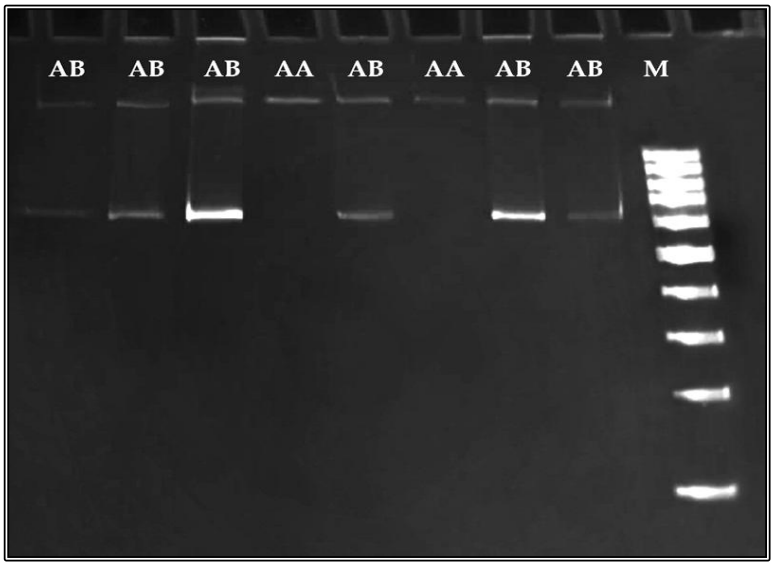

Fig. (1): Banding pattern of *MSTN* genotypes in V line using PCR-SSCP method on 10% PAGE. The genotypes are indicated at the top of each lane. M: (1000 bp) DNA marker (Promega, Germany).

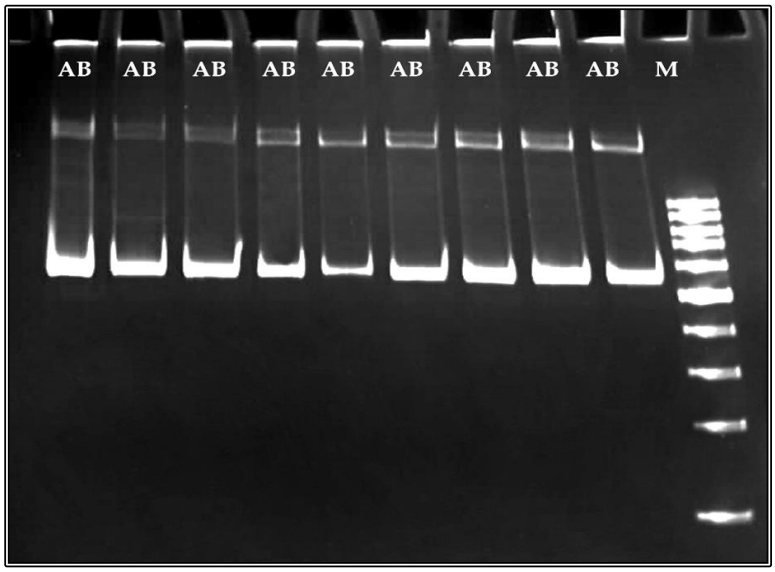

Fig. (2): Banding pattern of *MSTN* genotypes in Alex line using PCR-SSCP method on 10% PAGE. The genotypes are indicated at the top of each lane. M :(1000 bp) DNA marker (Promega, Germany).