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CORRELATING SINGLE NUCLEOTIDE POLYMORPHISMS IN THE MYOSTATIN GENE WITH PERFORMANCE TRAITS IN RABBIT

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Abstract: The Myostatin (MSTN), or Growth and Differentiation Factor 8 (*GDF8*), gene has been implicated in the double muscling phenomenon, in which a series of mutations render the gene inactive and unable to properly regulate muscle fibre deposition. Single nucleotide polymorphisms (SNPs) in the MSTN gene have been correlated to production traits, making it a candidate target gene to enhance livestock and fowl productivity. This study aimed to assess any association of three SNPs in the rabbit MSTN gene (c.713T>A in exon 2, c.747+34C>T in intron 2, and c.*194A>G in 3'-untranslated region) and their combinations, with carcass, production and reproductive traits. The investigated traits included individual body weight, daily body weight gain, carcass traits and reproductive traits. The 3 SNPs were screened using PCR-restriction fragment length polymorphism (RFLP)-based analysis and the effects of the different SNP genotypes and their combinations were estimated in a rabbit population. Additionally, additive and dominance effects were estimated for significant traits. The results found no significant association between the c.713 T>A SNP and all the examined traits. Allele T at the c.747+34C>T SNP was only significantly associated ($P<0.05$) with increased body weight at 12 wk of age. However, for the SNP residing in the 3' untranslated region (c.*194A>G), allele G was significantly associated ($P<0.05$) with increased body weight and high growth rate. Genotype GG at the c.*194A>G SNP also had positive effects on most carcass traits. The estimated additive genetic effect for the c.*194A>G SNP was significant ($P<0.05$) with most body weight, daily gain and carcass traits. No significant association was obtained between any MSTN SNPs and reproductive traits. In the combinations analysis, regardless of the genotypes of SNPs at c.713T>A and c.747+34C>T, GG at the c.*194A>G SNP correlated with highest values in body weight and daily weight gain. In conclusion, the 'G' allele at the c.*194A>G SNP had positive effects on growth and carcass traits and so could be used as a favourable allele in planning rabbit selection. Further population-wide studies are necessary to test the association of the c.*194A>G SNP with carcass traits. We also recommend evaluation of the potential effects of the c.*194A>G SNP on MSTN gene expression.

Key Words: rabbits, myostatin, SNPs, genotype, growth.

INTRODUCTION

The Myostatin (MSTN) or Growth and Differentiation Factor 8 (GDF8) gene has been implicated in a phenomenon known as double muscling, in which a series of mutations render the gene inactive and therefore unable to properly regulate muscle fibre deposition (Bellinge *et al.,* 2005). Polymorphisms in the MSTN gene have been correlated to production traits, making it a candidate target gene to enhance livestock and fowl productivity (Lu *et al.,* 2011; Han *et al.,* 2012 and Zhang *et al.,* 2012). However, in many cases, the allele associated with favourable production traits is not always favourable for reproductive traits. For example, some MSTN alleles are associated with longer postpartum

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anoestrous intervals in cows (Collis *et al.,* 2012), although this same allele had no influence on puberty age in heifers (Cushman *et al.,* 2015).

Rabbits exhibit several traits of economic importance, including a high rate of reproduction, early maturity, high growth rate and efficient feed utilisation (Bindu *et al.,* 2012). Due to these facts, we considered the rabbit MSTN gene as a candidate target gene to analyse for any correlations to meat production traits and, therefore, single nucleotide polymorphisms (SNPs) in the MSTN gene were studied.

Earlier studies had reported several SNPs in different regions of the rabbit MSTN gene. Fontanesi *et al.* (2008) sequenced the entire MSTN gene and detected one SNP, residing in intron 2 (c.747+34C>T). Later, Fontanesi *et al.* (2011) re-sequenced the MSTN gene, including the 3 coding exons, and identified 3 more SNPs in exon 1 (c.108C>T), exon 2 (c.713T>A), and in the 3'-untranslated region (UTR) (c.*194A>G). Kurkute *et al.* (2011) rescreened all 3 exons of the MSTN gene and found 2 mutations in exon 1 (c.1A>G) and exon 3 (c.799G>A). Peng *et al.* (2013) identified a T/C variant at -125 bp (relative to ATG start codon) in the rabbit MSTN gene, and, more recently, Sternstein *et al.* (2014) identified 2 novel SNPs (C.-125T>C) and (c. 373+234G>A) and confirmed one known SNP (c. 747+34C>T).

However, according to the available literature, few studies have investigated the effects of different SNPs in the rabbit MSTN gene on production traits. Fontanesi *et al.* (2011) reported that there was no significant association between 3 SNPs (c.713T>A, c.747+34C>T, and c.*194A>G) and weight at 70 d of age. Sternstein *et al.* (2014) reported a significant association between one reported SNP (c. 373+234G>A) and 9 carcass composition traits ($P<0.05$), while another 2 SNPs (c.125T>C and c.747+34C>T) had no significant associations. Until now, no correlation of MSTN SNPs with growth rates and reproductive traits in rabbits had been demonstrated. Therefore, the objective of this study was to estimate the association of 3 MSTN SNPs (c.713T>A, c.747+34C>T and c.*194A>G), alone and in combination, with productive, carcass and reproductive traits in the rabbit. Furthermore, we aimed to calculate the allele and genotype frequencies of these SNPs. These SNPs were chosen because they include different parts of MSTN gene: exons, introns and the UTR.

MATERIAL AND METHODS

Samples and Data Collection

APRI rabbits line used in this study were reared in the Animal Production Research Institute, Sakha, Kafr El-Sheikh governorate, Egypt. Sets of 2 growing rabbits were housed in one cage equipped with feeding hoppers made of galvanised steel with nipples for the automatic water supply. The rabbits were fed *ad libitum* on pelleted diet of 16.4% crude protein, 13.3% crude fibre with a digestible energy of 2500 kcal/kg diet. Blood samples were collected from 286 rabbits in K_2EDTA coated tubes, which were then stored at –20°C until DNA extraction.

We investigated productive, carcass and reproductive traits. The productive traits included body weight at 6, 8, 10 and 12 wk of age and daily body weight gain (DG) calculated at intervals of 6-8, 8-10 and 10-12 wk of age. These traits were recorded for a total of 286 rabbits; 86 males and 200 females produced from 62 sires. The carcass traits were taken for 54 males at 12 wk of age, at which point rabbits were slaughtered, skinned and eviscerated. The dressing percentage was calculated using the weight of edible giblets (WEG) and the cut-points of the carcass divided by live body weight and multiplied by 100. The variables measured for the carcass traits were WEG of edible giblets (total weights of liver, kidneys and heart) and carcass cuts. The reference carcass cuts according to Blasco *et al.* (1993) used the following points: Cut-point 1: Fore-quarter, section between the 7th and 8th thoracic vertebra, Cut-point 2: Intermediate, section between the last thoracic and the first lumbar vertebra, following the end of the 12th rib when cutting the thoracic wall, Cut-point 3: Loin, section after the 12th to before hind legs, and Cut-point 4: Hind-quarter, including hind legs and thighs.

The reproductive traits included the age of puberty (AP), number of services per conception (NSC) and kidding interval (KI). These traits were recorded for 200 females and 545 litters. All investigations were performed according to the rules accepted by the Local Commission for Ethics in Animal Experimentation Investigation on Animals.

DNA Extraction

A simplified protocol for the extraction of DNA from whole blood was used according to Darwish *et al.* (2013). Briefly, 500 µl of K_2EDTA blood was centrifuged at 3000 rpm for 10 min followed by supernatant removal and resuspension of the pelleted cells in TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8). Centrifugation, supernatant removal and resuspension steps were repeated until the cell pellet had lost all reddish colouration. The pellet was then resuspended in 100 µl of Chelex-100 (10%) followed by incubation at 100°C for 15 min. A final centrifugation step was then used to pellet the cell debris and the supernatant was transferred to clean tube and stored at –20°C to be used as a source for template DNA.

Genotyping using PCR-RFLP

Three SNPs (c.713T>A, c.747+34C>T and c.*194A>G) in the rabbit MSTN gene were screened using PCR-restriction fragment length polymorphism (RFLP)-based analysis according to Fontanesi *et al.* (2011). Table 1 shows the primer sequences, annealing temperatures, specific restriction enzymes and genotype identification for each SNP. Polymerase chain reactions (PCR) were carried out in 25 µl reaction volumes containing 5 µl template DNA, 20 pmol of each primer and 1 X Dream Taq Green PCR Master Mix (Fermentas Life Science). Amplification was carried out in a Nexus gradient Master cycler (Eppendorf, Germany) under the following conditions: Initial denaturation for 5 min at 95°C, 35 amplification cycles of 30 s at 95°C, 30 s at the appropriate annealing temperature (as shown in Table 1), 30 s at 72°C followed by 10 min at 72°C for final extension. Digestion of PCR products using specified restriction enzymes (as shown in Table 1) was performed according to manufacturing manuals (Fermentas, Life Sciences). Digestion fragments patterns were screened by electrophoresis using a 3% agarose gel in 1 X TBE electrophoresis buffer and were visualised with ethidium bromide on a UV-transilluminator as shown in Figures 1A-C. We note that the digestion pattern of the MSTN c.713T>A SNP was different from that reported by Fontanesi *et al.* (2011). They reported the T allele to give an undigested band of 570 bp while the A allele gives 2 bands of 445 and 125 bp. Our results in Figure 1A demonstrated that the T allele gave 2 bands of 500 bp and 70 bp while the A allele gave 2 bands of 450 bp and 70 bp. This may be explained by the presence of another restriction site for the used enzyme and occurred for all genotypes. Regardless of this finding, according to the 3 genotypes, rabbits were classified into 3 groups for association studies.

Table 1: SNPs, PCR primers, amplified fragments, annealing temperatures, specified restriction enzymes and genotyping identification keys.

	Primer pair names		
	MSTN SNP c.713T>A	*MSTN* SNP c.747+34C>T	*MSTN* SNP c.*194A>G
Forward and *reverse* primers (5'-3')[1]	TGCATGCATTATCCCAATAGA TCGGTAGTTGTTTCCCACTTT	TAACTGAAAAGAACCCTCTAGTAGC TCGGTAGTTGTTTCCCACTTT	TACATGCAATGGTTGGCATT TCAGAACACAAGGAGAATTGC
Gene regions[2]	Part of intron 1, exon 2 and part of intron 2	Part of intron 2	3' untranslated region
T (°C)	57	55	57
Amplified fragments	570 bp	80 bp	158 bp
Restriction enzyme	*Fsp*BI	*Alu*I	*Taa*I
Genotyping[3]	allele T=570 bp, allele A=445+125 bp	allele C=80 bp; allele T=56+24 bp	allele A=158 bp; allele G=115+43 bp

[1] Primers were designed using the Ensembl ENSOCUG00000012663 rabbit gene genome by Fontanesi *et al.,* 2011.
[2] The sequenced rabbit *MSTN* regions.
[3] Genotyping was carried out by PCR-RFLP.
T: Annealing temperature.

Statistical Analysis

Allele and genotype frequencies of each SNP polymorphism, in addition to their deviation from Hardy-Weinberg equilibrium using χ^2 test, were calculated using the Online Encyclopedia for Genetic Epidemiology studies (OEGE) on the American Journal of Epidemiology website (http://www.oege.org/software/hardy-weinberg.html).

The effects of genotypes of different SNPs on different traits were estimated using the SAS mixed procedure version 9.2 (SAS, 1999) with a model in equation 1:

$$Y_{ijklmno}=\mu + P_i + S_j + Sex_k + Dam_l + MSTN_m + Sire_n + Dam_o(Sire_n) + b*LS + e_{ijklmno,} \quad (1)$$

where $Y_{ijklmno}$=any observation of animals in test; μ=overall mean; P_i=fixed effect of the i^{th} parity (3 classes 1, 2 and ≥3); S_j=fixed effect of the j^{th} Season (4); Sex_k= fixed effect of Sex (2); $MSTN_l$=fixed effect of the l^{th} MSTN genotypes (3) for each separated SNP; $Sire_m$=random effects of Sire (62); Dam_o=random effects of dam (143) nested within Sire; LS=the covariate litter size at birth (7 classes ≤4,5,6,7,8,9,10 and ≥11); $e_{ijklmno}$=residual error.

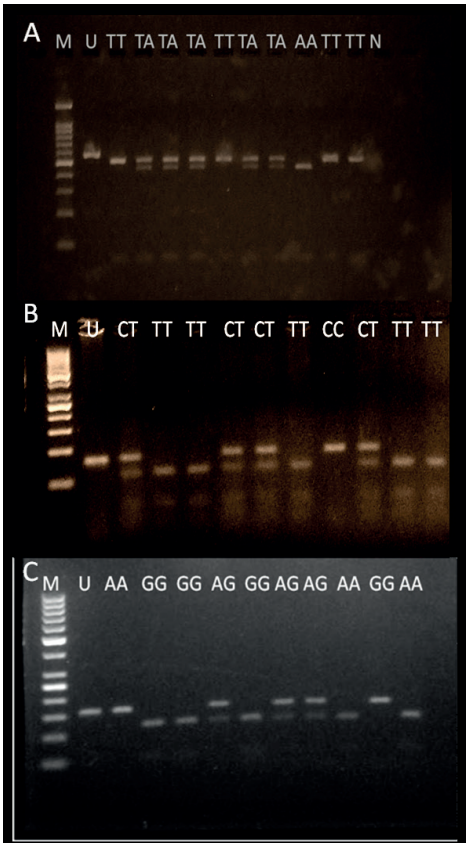

Figure 1: A) SNP (c.713 T>A) genotypes. M: 100 bp ladder DNA marker, U: control PCR product (570 bp). B) SNP (c.747+34C>T) genotypes. M: 50 bp ladder DNA marker, U: control PCR product (80 bp). C) SNP (c.194A>G) genotypes. M: 50 bp ladder DNA marker, U: control PCR product (158 bp). Different genotypes of each SNP were shown on each figure.

Additive and dominance effects were estimated according to Russo *et al.* (2008). The additive and dominance effects on significant deviation from zero were tested by the t-test. Any additive genetic effect (a) for each SNP genotype was estimated as half of the difference between values of the 2 homozygous groups according to the equation: a=½(pp–qq). The dominance effect (d) at each locus was estimated as the difference between the values of the heterozygous group and the average of the values of the 2 homozygous groups according to the equation: d=pq–½(pp+qq). Combinatorial effects of the 3 MSTN SNPs on different traits were estimated in the rabbit population using the SAS mixed procedure version 9.2 (SAS, 1999), with the same model as in equation 1.

RESULTS AND DISCUSSION

All rabbit samples were genotyped for the 3 SNPs using the PCR-RFLP method. Table 2 shows the alleles and genotype frequency of each SNP. The rabbit population displayed both alleles at the c.713T>A SNP, with A representing the minor allele (Table 2). Both alleles in the SNPs at c.747+34C>T and c.*194A>G were detected (Table 2). The alternative alleles T at the c.747+34C>T and G at the c.*194A>G SNP were the major alleles, with C and A the minor alleles (Table 2). Fontanesi *et al.* (2008) reported the frequencies of the C and T alleles of SNP at c.747+34C>T to be 0.51 and 0.49, respectively, in their rabbit population (commercial paternal line). The same alleles (T and C alleles at c.747+34C>T) displayed frequencies of 0.67 and 0.33, respectively, in M91 and P91 rabbit lines (Rafayova *et al.,* 2009) and in Giant Grey and New Zealand White rabbit (Sternstein *et al.,* 2014). These frequencies are similar to those found in this study. However, the T and C allele frequencies were reported at 0.38 and 0.62, respectively, in Polish and White Flemish

Table 2: Allele frequency and observed and expected genotype frequencies for rabbit *MSTN* SNPs and chi-square (χ^2) values for Hardy-Weinberg equilibrium test at $P<0.05$.

Position	Allele frequency			Genotyping data			Total
Exon 2	Allele T	Allele A		TT	AA	TA	
(c.713T>A)	0.73	0.27	Observed	150	20	114	284
	χ^2=0.07		Expected	150.88	20.88	112.25	
Intron 2	Allele T	Allele C		TT	CC	CT	
(c.747+34C>T)	0.73	0.27	Observed	156	22	108	286
	χ^2=0.3		Expected	154.21	20.22	111.61	
3'-UTR	Allele G	Allele A		GG	AA	AG	
(c.194A>G)	0.72	0.28	Observed	148	23	110	281
	χ^2= 0.16		Expected	146.65	21.65	112.7	

Giants Rabbits (Markowska *et al.,* 2010) and 0.43 and 0.57 in a pooled population of New Zealand White and Soviet Chinchilla and their crosses (Bindu *et al.,* 2012).

The number of observed and expected genotypes of MSTN SNPs (c.713T>A, c.747+34C>T and c.*194A>G) are shown in Table 2. Chi-square (χ^2) values indicated that the numbers for the expected and the observed genotypes were very close and the population was considered to be in Hardy-Weinberg equilibrium. This could be attributed to the high number of heterozygous genotypes that may maintain the balanced allele frequency in a population. Increasing the number of heterozygous genotypes may be a logical next step and agrees with the recent production of the APRI rabbit line (Youssef *et al.,* 2008).

No significant correlations were observed between the MSTN c.713T>A SNP and the traits examined (individual body weight, daily body weight gain, carcass traits and reproductive traits) as shown in Table 3. These results are

Table 3: Least square means ± standard error of investigated traits for rabbit *MSTN* SNPs (c.713 T>A) genotypes.

	TT	AA	TA		
Body weight & body gain:	n=150	n=20	n=114	Additive effect	Dominance effect
BW6	689.5±36.0	675.4±54.3	713.5±41.5	7.00	30.1
BW8	902.0±46.8	856.4±61.5	895.3±53.9	22.80	16.1
BW10	1144.6±48.4	1086.1.3±67.1	1168.4±55.8	29.27	51.0
BW12	1405.8±54.5	1423.8±88.3	1432.0±62.8	–9.00	17.5
DG6-8	15.3±1.9	14.1±3.6	13.1±2.2	0.60	–1.6
DG8-10	16.1±1.4	15.9±3.1	17.7±1.7	0.10	1.6
D10-12	18.8±2.2	19.1±3.8	18.4±2.6	–0.15	–0.53
% carcass cuts:	n=25	n=5	n=24		
WEG (%)	4.53±0.17	4.52±0.45	4.64±0.17	0.01	0.11
Fore-quarter	11.76±0.22	11.30±0.57	11.78±0.21	0.22	0.25
Intermediate	5.48±0.16	6.00±0.43	6.19±0.16	–0.26	0.45
Loin	7.84±0.26	7.56±0.67	7.70±0.25	0.14	0.01
Hind-quarter	18.69±0.25	18.20±0.65	19.35±0.24	0.24	0.90
Reproductive traits:	n=106	n=13	n=81		
AP (wk)	32.4±1.32	26.3±4.88	33.4±1.57	3.0	4.0
KI	54.9±10.0	68.2±6.6	38.9±9.0	–6.6	–22.4
NSC	1.56±0.06	1.36±0.18	1.48±0.07	0.10	0.02

BW: body weight at 6, 8, 10 and 12 wk; DG: Daily body gain; WEG: weight of edible giblets; AP: Age of puberty by day; KI: Kindling interval; NSC: no. of services per conception.
Additive effect=½(TT–AA); dominant effect=TA–½(TT+AA).

in agreement with the findings of Fontanesi *et al.* (2011) and confirmed that the c.713T>A SNP in exon 2 is a synonymous mutation. In contrast to Fontanesi *et al.* (2011), who could not find any association between an MSTN SNP (c.747+34C>T) and final weight, our study provides evidence for a significant association ($P<0.05$) of this SNP with body weight of rabbits (Table 4). Allele T was associated significantly ($P<0.05$) with increased body weight at 12 wk of age, although no significant association was found between this SNP and the daily weight gain (DG) during intervals of the growth or carcass traits (Table 4). Results of the carcass traits (Table 4) agree with the findings of Sternstein *et al.* (2014) who reported no significant effects for the c.747+34C>T SNP on nine carcass composition traits in the rabbit. The estimated additive genetic effect (a) in the population was significant ($P<0.05$) with body weight at 12 wk only (Table 4). This may be supported by a positive effect for allele T on body weight, which increased by 104 g at 12 wk of age.

Regarding the SNP in the 3' UTR (c.*194A>G), allele G was significantly associated ($P<0.05$) with increased body weight and high growth of rabbits during development (Table 5). Allele G was associated with increased body weight values at 10 and 12 wk, as those carrying the GG genotype weighed 114.3 and 149.7 g higher weight than those with the AA genotype, respectively. Body weight at 10 wk showed higher correlations (r=0.299, 0.460 and 0.689, $P<0.05$) than with weight at 6, 8 and 12 wk. The DG within the period of 8 to 10 wk of age showed high correlation (r=0.5480 and 0.5236) to body weight at 10 and 12 wk. The GG genotype of the c.*194A>G SNP had positive effects on percentage WEG, hind-quarter and intermediate weights, and a negative effect on the percentage of fore-quarter weights (Table 5). In contrast, genotype AA had the opposite effect. In the rabbit, the major fat deposits are found in the abdomen and shoulders (Blasco *et al.,* 1993 and Maertens and De-Groote, 1992), which are part of the fore-quarter and loin cuts, respectively. It was postulated that inheriting the 'G' allele of this SNP display would increase skeletal muscle and bone weight and increase all carcass traits values, except the intermediate part. These findings may support MSTN as a candidate gene related to muscle growth and development (Koohmaraie 1996; Knapp *et al.,* 2006). In the present study, the hind-quarter was highly correlated (r=0.681) with the intermediate cut, although there was a negative correlation with the fore-quarter and loin cuts (r=–0.536 and -0.659, respectively). No significant association was obtained between the c.*194A>G SNP and reproductive traits, including age of puberty by day (AP), kindling interval (KI) and number of services per conception (NSC) as shown in Table 5. Cushman *et al.* (2015) reported an MSTN polymorphism to be significantly associated with a delay in the onset of puberty in cattle

Table 4: Least square means±standard error of investigated traits for rabbit *MSTN* SNPs (c.747+34C>T).

	CC	TT	CT		
Body weight & body gain:	n=22	n=156	n=108	Additive effect	Dominance effect
BW6	627.1±95.7	680.8±42.8	646.8±63.7	–26.9	–6.4
BW8	832.5±59.8	849.2±26.7	845.4±39.8	–8.2	4.6
BW10	1074.6±97.6	1141.5±48.2	1105.1±71.7	–33.4	–2.9
BW12	1282.2±75.3^{a}	1386.2±50.0^{b}	1356.8±67.3^{ab}	–52.0*	22.3
DG6-8	12.6±4.6	12.4±2.0	12.0±3.1	0.13	–0.20
DG8-10	13.4±4.3	14.9±2.9	14.2±3.9	–0.75	0.04
D10-12	15.4±5.5	17.3±3.7	15.7±2.4	–0.91	–0.62
% carcass cuts:	n=4	n=29	n=21		
WEG (%)	4.60±0.39	4.63±0.15	4.47±0.21	–0.02	–0.14
Fore-quarter	11.85±0.50	11.65±0.19	11.87±0.26	0.10	0.12
Intermediate	5.32±0.41	5.90±0.16	5.91±0.21	–0.29	0.30
Loin	7.62±0.58	7.69±0.22	7.92±0.31	–0.04	0.26
Hind-quarter	18.42±0.59	18.95±0.23	19.1±0.31	–0.26	0.41
Reproductive traits:	n=16	n=109	n=75		
AP(wk)	31.0±2.78	31.2±1.35	35.1±1.63	–0.10	4.0
Days Open	56.5±14.2	64.8±5.4	38.2±9.3	–4.15	–22.4
NSC	1.33±0.14	1.54±0.06	1.53±0.09	–0.11	0.09

BW: body weight at 6, 8, 10 and 12 weeks; DG: Daily body gain; WEG: weight of edible giblets; AP: Age of puberty by day; KI: Kindling interval; NSC: no. of services per conception. Different letters in the same row mean significant differences at $P<0.05$. Additive effect=½(CC–TT); dominant effect=CT–½(CC+TT). * Significant at $P<0.05$.

Table 5: Least square means±standard error of investigated traits for rabbit *MSTN* SNPs c.*194A>G.

	AA	GG	AG		
Body weight & body gain:	n=23	n=148	n=110	Additive effect	Dominance effect
BW6	647.1±87.3	689.8±47.1	643.5±61.6	–21.3	–24.9
BW8	838.2±51.9	853.9±28.0	842.1±36.6	–7.8	–3.9
BW10	1055.1^{a}±81.5	1169.4^{c}±44.0	1103.1^{b}±57.5	–57.1*	–9.0
BW12	1270.8^{a}±92.7	1420.5^{b}±78.7	1354.5^{b}±67.9	–74.8*	8.7
DG6-8	12.4±4.1	12.1±2.2	12.2±2.9	0.15	–0.02
DG8-10	11.8^{a}±1.9	15.9^{b}±1.5	14.3^{b}±2.0	–2.0*	0.45
D10-12	13.5^{a}±2.0	18.7^{b}±1.6	15.6^{a}±1.4	–2.6*	–0.47
% carcass traits:	n=9	n=24	n=21		
WEG (%)	4.19^{a}±0.18	5.12^{b}±0.16	4.38^{a}±0.16	–0.47*	–0.28
Fore-quarter	12.22^{b}±0.24	11.08^{a}±0.21	11.94^{b}±0.21	0.57*	0.29
Intermediate	5.28^{a}±0.21	6.24^{b}±0.18	5.92^{ab}±0.18	–0.48*	0.16
Loin	8.14±0.27	7.50±0.23	7.54±0.23	0.32	–0.28
Hind-quarter	17.90^{a}±0.28	19.56^{b}±0.23	19.14^{b}±0.24	–0.83*	0.41
Reproductive traits:	n=12	n=109	n=79		
AP(wk)	31.9±1.99	31.5±1.54	34.3±1.69	0.20	2.60
Days Open	52.2±6.5	83.0±14.7	58.4±7.9	–15.40	–9.20
NSC	1.59±0.09	1.45±0.07	1.53±0.06	0.07	0.01

BW: body weight at 6, 8, 10 and 12 weeks; DG: Daily body gain; WEG: weight of edible giblets; AP: Age of puberty by day; KI: Kindling interval; NSC: no. of services per conception. Different letters in the same row mean significant differences at $P<0.05$. Additive effect=½(AA–GG); dominant effect=AG–½(AA+GG). *Significant at $P<0.05$.

while no significant effects were observed in pregnancy rates among the breeds of heifers that were evaluated (Cundiff *et al.,* 2007 and Cushman *et al.,* 2015). The cause of this is not clear because the mechanism of action of the MSTN polymorphism is unknown.

The estimated additive genetic effect (a) for the c.*194A>G SNP was significant ($P<0.05$) with body weight at weeks 10 and 12 and daily gain at DG 8-10 and DG 10-12, as shown in Table (5). These results indicate that part of the

Table 6: Least square means (LSM)±standard error (SE) of body weight (BW) and daily gain (DG) assessed in relation to the combination of 3 SNPs in the rabbit *MSTN* gene.

	Genotype	TT/CC/ AA	TT/TT/AA	TT/TT/ GG	TT/CT/ AG	AA/TT/ GG	AA/TT/ AG	TA/TT/ GG	TA/CT/ AG
Traits	n=269*	n= 14	n=9	n= 66	n=56	n=6	n=6	n=64	n= 48
BW6	LSM	624.9^{a}	654.2^{ab}	677.9^{ab}	653.4^{ab}	678.2^{ab}	670.0^{ab}	707.2^{b}	636.9^{ab}
	±SE	25.5	23.4	13.3	11.8	29.9	23.4	12.4	14.1
BW8	LSM	833.0	838.3	844.3	852.4	821.5	824.3	864.3	826.7
	±SE	28.4	26.1	14.9	13.2	33.3	26.1	13.9	15.7
BW10	LSM	1087.9^{ab}	1025.6^{a}	1158.6^{bc}	1104.8^{abc}	1132.2^{abc}	1047.0^{a}	1197.3^{c}	1098.1^{abc}
	±SE	42.5	39.1	22.3	19.7	49.9	39.1	20.8	23.5
BW12	LSM	1273.3^{b}	1233.6^{b}	1419.9^{c}	1377.5^{bc}	1446.8^{c}	1260.3^{b}	1428.8^{c}	1318.0^{bc}
	±SE	58.2	53.5	30.5	27.0	68.2	53.5	28.4	32.1
DG6-8	LSM	13.5	12.4	13.2	13.4	11.4	10.7	11.1	10.5
	±SE	1.78	1.64	0.93	0.82	2.09	1.64	0.87	0.98
DG8-10	LSM	14.0^{b}	9.3^{a}	16.3^{b}	13.7^{ab}	17.4^{b}	12.6^{ab}	16.0^{b}	14.9^{b}
	±SE	1.92	1.77	1.01	0.89	2.26	1.77	0.94	1.06
DG10-12	LSM	15.3^{ab}	12.2^{a}	18.9^{b}	16.4^{ab}	18.6^{b}	13.2^{ab}	18.9^{b}	14.6^{ab}
	±SE	2.39	2.20	1.25	1.11	2.80	2.20	1.17	1.32

*Only 8 combinations were studied due to very low frequency of the other 8 combinations.
Means not sharing superscript were significantly different at $P<0.05$.

Table 7: Least square means (LSM)±standard error (SE) of carcass of traits assessed in relation to the combination of 3 SNPs in the rabbit *MSTN* gene.

% carcass traits:		TT/TT/AA	TT/CC/GG	TT/TT/GG	TT/CT/AG	AA/TT/GG	TA/TT/GG	TA/CT/AG
	n=54	n=9	n= 4	n=6	n=6	n= 5	n=9	n=15
WEG (%)	LSM	3.8^{a}	4.8^{b}	5.8^{c}	4.3^{ab}	4.7^{ab}	5.2^{bc}	4.5^{ab}
	±SE	0.23	0.33	0.29	0.29	0.33	0.23	0.18
Fore-quarter	LSM	12.2	11.9	10.9	11.7	10.9	10.9	11.9
	±SE	0.37	0.52	0.45	0.45	0.52	0.37	0.28
Intermediate	LSM	4.8^{a}	5.2^{a}	6.5^{b}	5.0^{a}	6.3^{b}	6.5^{b}	6.2^{b}
	±SE	0.22	0.32	0.27	0.27	0.32	0.22	0.17
Loin	LSM	8.2	8.1	7.3	8.6	7.4	7.3	7.6
	±SE	0.47	0.67	0.58	0.58	0.67	0.47	0.36
Hind-quarter	LSM	18.2^{a}	18.4^{ab}	19.8^{c}	18.4^{ab}	19.7^{c}	19.8^{c}	19.4^{bc}
	±SE	0.32	0.45	0.39	0.39	0.45	0.32	0.24

WEG: weight of edible giblets. Means not sharing superscript were significantly different at $P<0.05$.

genetic variance in these traits was affected more by the 'G' allele than the 'A' allele. No significant dominance effects were observed for the c.*194A>G SNP on the investigated traits (Table 5).

Considering the combinations of the 3 MSTN SNPs, 16 types of combinations were found but 8 types were excluded because they were present in only 1-4 rabbits. The genotype GG had significant positive effects when compared to AA at c.*194A>G on most of the investigated traits. Regardless of genotypes of SNPs at c.713T>A and c.747+34C>T, the GG or AG genotypes of the c.*194A>G SNP were associated with highest values for body weight and daily weight gain (Table 6) and carcass traits (Table 7). These association results agreed with the effects of allele 'G' in the SNP at c.*194A>G as shown in Table 5. The frequencies of rabbits which had GG and AG genotypes as the favourable alleles were high (Table 6). This could be attributed to the fact that APRI rabbit line was originally a crossing between Egyptian breed (Baladi Red) and Spanish line (V-line) and was selected for litter weight at weaning (Youssef *et al.,* 2008).

According to the available literature, this study is the first to report an association of variation at 3 SNPs (c.713T>A, c.747+34C>T, and c.*194A>G) in the MSTN gene with body weight and daily gain during the growing period of rabbits. However, the functional effect of the c.*194A>G SNP in the 3'UTR is unclear. The c.*194A>G SNP does not affect the protein sequence, but may have a regulatory influence on gene expression. Furthermore, a regulatory influence on post-transcriptional processes (Neilson and Sandberg, 2010) and protein expression cannot be ruled out (Sauna and Kimchi-Sarfaty, 2011). Conne *et al.,* (2000) reported that non-coding SNPs harbour potentially important DNA sequence variants influencing phenotypes in mammals. In sheep, Clop *et al.* (2006) reported a mutation in the 3'UTR of MSTN to be associated with muscular hypertrophy. This mutation was reported to create a target site for microRNAs highly expressed in skeletal muscle and to cause translational inhibition of the MSTN gene. All of these examples could explain the mechanism involved in the effect of allele G in the c.*194>G SNP in the UTR. Additionally, we note that this positive association may be due to a further causative mutation or to a linkage disequilibrium between this mutation and another unknown causative mutation.

CONCLUSIONS

The 'G' allele at the c.*194A>G SNP had positive effects on growth performance and carcass traits and may be used as a favourable allele in selection of rabbits. Moreover, this SNP did not produce any negative effects on the reproductive traits tested. Further population-wide studies may be necessary to test the association of the c.*194A>G SNP detected in the 3'UTR of the MSTN gene with carcass traits. Additionally, evaluation of the potential effects of c.*194A>G SNP on the expression of MSTN gene in the rabbit may also be necessary.

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