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SNPs of the *MyoD* and *MyoG* genes and their association with growth traits in Egyptian water buffalo *(Bubalus bubalis)*

KEYWORDS: *MyoD* gene, *MyoG* gene, Egyptian buffalo, Sequencing, SSCP, SNPs, Average daily gain

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ABSTRACT *Myogenic determination gene (MyoD) and Myogenin (MyoG) are members of the myogenic regulatory factors (MRF) gene family which have great role in the development of skeletal muscle. Association between the polymorphisms of MyoD and MyoG genes and growth traits were studied in many species but have not elucidated yet in buffalo. Therefore, the aim of this study was to detect polymorphisms of these two genes, their allele, and genotype frequencies and to determine associations between these polymorphisms and growth traits in Egyptian water buffalo. Four loci of the MyoD gene and five loci of MyoG gene were amplified by PCR and subsequently, subjected to single strand conformation polymorphism (SSCP) followed by sequence analysis to identify different allelic patterns. A total of 4 novel single nucleotide polymorphisms (SNPs) were detected among Egyptian water buffaloes; A1481C SNP in intron 2, C1876T SNP in 3/UTR of MyoD and T1198C in intron 1, C2858T in 3/UTR of MyoG. Moreover, 23 SNPs in MyoD were determined in Egyptian water buffaloes as compared to Italian buffalo (FJ194946), and 43 SNPs in MyoG compared with water buffaloes in China (EF636460). The association of these polymorphisms with growth traits in Egyptian buffalo was analyzed using the general linear model (GLM) which revealed no significant association of any detected SNP with the measured growth traits from birth to 24 mo of age. Thus, these 4 SNPs may not influence growth traits in Egyptian buffalos.*

Introduction

Water buffaloes (*Bubalus bubalis*) could be taken as a promising alternative to crossbred cattle due to its great adaptability to varied ecological conditions (Fatima et al., 2009; Michelizzi et al., 2010), and its ability to convert poor roughages into more valuable products like milk and meat (Franzolin, 1994; Paul et al., 2003; Bilal et al., 2006). The buffalo meat is lean, tender, juicy, highly palatable and so it is more acceptable for consumption (Spanghero et al., 2004). Meat production, carcass quality and growth traits are controlled by some genes (markers) related to muscle development and growth. These genes collectively called myogenic genes. The myogenic regulatory factors *(MRF)* gene family includes the majority of these myogenic genes. Myogenic determination gene (*MyoD*) and Myogenin (*MyoG*), as two important members of this family, have great role in the development of skeletal muscle. In cattle, the *MyoD* and *MyoG* genes are located on chromosome 15 (BTA15) and chromosome 16 (BTA16), respectively (Beever et al., 1997; Ryan et al., 1997). In buffalo, the *MyoD* and *MyoG* genes are localized on chromosome 16 (BBU16) and chromosome 5 (BBU5), respectively (Strazzullo et al., 2010). The *MyoD* and *MyoG* genes consist of three exons and two introns (Ensembl database, 2013). *MyoD* plays a key role in the growth and development of skeletal muscle, and regulation of phenotypic traits (te Pas, 2004; Berkes and Tapscott, 2005; Liu et al., 2008; Bhuiyan et al., 2009; Ujan et al., 2011a; Wu et al., 2012). *MyoD* expression is sufficient to convert a fibroblast to a skeletal muscle cell (reviewed by Tapscott, 2005). *MyoG* is involved in the early stages of skeletal muscle differentiation and regeneration (Berkes and Tapscott, 2005). *MyoG* is a candidate gene responsible for muscle fibre characteristics (Kim et al., 2009) and the most pronounced effect on meat quality is exerted by this gene (Kapelanski et al., 2005).

As a result of *MyoD* and *MyoG* action in early development and growth of muscle, some studies had investigated the SNPs of the two genes and their effect on carcass quality and animal growth. In bovine *MyoD*, A1274G SNP in intron 1 and C39T and C112G SNPs in intron 2 were shown to be associated with live weight, carcass weight, and growth traits (Tian et al., 2007; Bhuiyan et al., 2009). In bovine *MyoG*, 4 SNPs in exon 1: G48C (Wang et al., 2011), T201G (Bhuiyan et al., 2009), T314C (Xue et al., 2011) and A959G (Ujan et al., 2011b), have significant associations with animal growth traits and carcass quality. In pig, a G174C SNP in 3′UTR of *MyoG* is associated with birth weight, growth rate, and carcass quality (Soumillion et al., 1997; te Pas et al., 1999; Cieslak et al., 2000; Kapelanski et al., 2005; Verner et al., 2007; Stupka et al., 2012). Some SNPs are detected in the *MyoG* gene of some other animals but their statistical analysis showed no correlation with carcass quality and/or growth traits; G237A and G46A SNPs in deer (Song et al., 2010) and C558T SNP in goat (Liu et al., 2011).

To date, the only association study in water buffaloes, detected two synonymous A5T and T201G SNPs in exon 1 of *MyoG* but did not find any effect on the growth traits (Wang et al., 2011) and until this study there is no investigation was carried out on *MyoD* SNPs in buffalo. Therefore the aim of this study is to detect novel SNPs in *MyoD* and *MyoG* genes and to study their association with average growth traits in Egyptian buffalo.

Material and methods

Animal source

Animal source was from El-Nataff El-Gidid Experimental Stations, Mahalet Mousa, Kafrelsheikh Governorate. The 200 animals used in this study were chosen at random. These

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animals were pure Egyptian water buffaloes, based on farm records and animal appearance. Moreover, these animals were artificially inseminated by fresh semen collected from buffalo studs on the farm. All records of body weight, at different growth periods (at birth and at 3, 6, 9, 12, 18, and 24 mo of age) were collected from the farm records.

DNA extraction
Blood samples were collected from 200 buffaloes by jugular vein puncture into vacutainer tubes containing an anticoagulant (disodium EDTA) and kept in ice box and then stored at −45°C. Genomic DNA was extracted from blood using Gene JET genomic DNA extraction kit following the manufacturer protocol (Fermentas, #K0721, European Union).

Polymerase chain reaction (PCR)
Four loci of *MyoD* gene and five loci of *MyoG* gene encompassing the whole sequences of both genes in Egyptian buffalo were amplified by PCR using nine pairs of overlapped primers (Table 1) designed by Primer 5.0 software based on the published nucleotide sequence of Italian buffalo *MyoD* (GenBank accession FJ194946) and Chinese buffalo *MyoG* (GenBank accession EF636460). The PCR was carried out in a reaction volume of 50 µL, containing 4.0 µL DNA template (approximately 100 ng), 1.0 µL (0.20 mM) dNTP, 5.0 µL buffer, 3.0 µL (2.5 mM) MgCl2, 2.0 µL 10µmol/L forward primer, 2.0 µL 10µmol/L reverse primer, 1.0 µL 10X Taq DNA polymerase (5 U/µL, Fermentas, #K1071, European Union), and 32.0 µL nuclease free water. Thermal cycling parameters were as follows: 1st system [initial denaturation at 94°C for 5 min, 35 cycles of amplification (94°C for 40 s for DNA denaturation, annealing temperatures as seen in table (1) for 1 min, extension at 72°C for 1 min) and final extension at 72°C for 10 min for (*MyoD*.1, .2, .3, *MyoG*.1, .2 and .3)] and the 2nd system [initial denaturation at 94°C for 10 min, 35 cycles of amplification (94°C for 30 s for DNA denaturation, annealing temperatures as seen in table (1) for 45 s, extension at 72°C for 1 min) and final extension at 72°C for 10 min for (*MyoD*.4, *MyoG*.4 and .5)]. The samples were held at 4°C. PCR products were subjected to electrophoresis on 1.5% agarose gels using 1X TAE buffer containing 0.2 pg ethidium bromide per ml.

Single stranded conformation polymorphism (SSCP) and sequencing
PCR products were resolved by SSCP analysis, 5 µL PCR products were mixed with 5 µL denaturing solution (25 mM EDTA, 95% formamide, 0.025% bromophenol blue and 0.025% xylene-cyanole), heated for 10 min at 94°C and chilled on ice and then loaded on a nondenaturing 10, 12 and 14% polyacrylamide gels (39:1 acrylamide to bis-acrylamide). After electrophoresis, which was performed in 1X TBE buffer at 200 V for 10 – 14 hours at 4°C, the DNA fragments in the gel were stained with a 0.5 µg/ml solution of ethidium bromide in 1X TBE buffer for 20 min, and then destained in distilled water for 5 min. SSCP patterns were identified by differential migration because of fragment conformation. The PCR products of different electrophoresis patterns were purified and then sent to MacroGen Company (South Korea) to sequence in both directions using ABI 3730XL DNA sequencer (Applied Biosystem, USA). Geneious 4.8.4 software was used to analyze sequences.

Statistical analysis
Genotype and allele frequencies of A1481C SNP in intron 2 and C1876T SNP in 3′UTR in *MyoD*, T1198C SNP in intron1 and C2858T SNP in 3′UTR in *MyoG* were directly estimated in Egyptian buffaloes. The Hardy–Weinberg (H–W) equilibrium was assessed by applying the X^2 test. Statistical analyses were conducted using least square means estimates (LSM) procedure by using SPSS (version 16.0) software to analyze the relationship between genotypes of the detected SNPs and growth traits in buffalo according to the following linear model: $Y_{klm} = \mu + A_k + G_l + E_{klm}$, where Y_{klm} was the trait measured on each of the klm^{th} animals, µ was the overall population mean, A_k was fixed effect due to the k^{th} age (in days), G_l was the fixed effect associated with l^{th} genotype and E_{klm} was the random error.

3. Results
MyoD clones were determined using PCR (Fig.1) and different genotypes of three *MyoD* loci were identified in 200 Egyptian buffaloes using PCR-SSCP method conditioning three banding patterns (AA/AC/CC) in *MyoD.3* locus (Fig.2A) and (CC/CT/TT) in *MyoD.4* locus (Fig.2B). The sequence of *MyoD* in Egyptian water buffalo (submitted to GenBank with accession number KC107776) showed two SNPs; A1481C at nucleotide number 137 of intron 2 (Fig.2C), C1876T at nucleotide number 19 of 3′UTR (Fig.2D) among the Egyptian buffaloes and 23 SNPs in compared with Italian water buffalo (FJ194946) (Table 2).

Five loci, *MyoG*.1-5, encompassing the whole sequences of the *MyoG* gene were determined using PCR (Fig. 3) and their genotypes were identified in 200 Egyptian buffaloes using SSCP method which revealed two SSCP banding patterns (TC and CC) in *MyoG.3* locus (Fig. 4A) and (CT and TT) in *MyoG.5* locus (Fig. 4B). The whole sequence of the *MyoG* gene in Egyptian water buffalo was submitted to GenBank with accession number KC107779. This sequence showed two SNPs; T1198C SNP at nucleotide number 556 of intron 1 (Fig. 4C), and a C2858T SNP at nucleotide number 625 of 3′UTR (Fig. 4D) among the Egyptian buffaloes and 43 SNPs compared with buffalo in China (EF636460) (Table 3).

The genotypic frequencies and the allele frequencies were calculated for the four SNPs (Table 4). All genotypes were in Hardy–Weinberg equilibrium ($P > 0.05$), as shown in Table 4.

Association analysis between the different genotypes of the four novel SNPs (A1481C, C1876T SNPs in *MyoD*, and T1198C, C2858T SNPs in *MyoG*) and the growth traits (body weight and average daily gain, ADG) in Egyptian buffaloes at birth and at 3, 6, 9, 12, 18, and 24 mo of age showed no significant association (Tables 5 - 8).

Discussion
In past before the advent of molecular genetics, phenotype selection was widely used by breeders to improve animal productivity. There is a practical limitation of phenotypic selection which does not significantly improve growth traits. In the recent years, molecular genetics and biotechnology have been successfully used to improve the meat production of farm animals. Candidate genes and comparative mapping approaches had been successful in identifying major genes affecting several traits (Rejduch, 2008). *MyoD* and *MyoG* influence the development of skeletal muscles. They act as key transcription factors for myogenesis (Ishido et al., 2004; Sung et al., 2013) and considered as candidate genes for meat production and growth traits in farm animals (te Pas, 2004; Xue and Zhou, 2006; Tian et al., 2007; Zhang et al., 2007; Bhuiyan et al., 2009; Liu et al., 2011; Ujan et al., 2011b; Yin et al., 2011; Lee et al., 2012; Stupka et al., 2012). In the present study, a total of 4 novel SNPs; A1481C SNP in intron 2, C1876T SNP in 3′UTR of *MyoD* and T1198C SNP in intron 1, C2858T SNP in 3′UTR of *MyoG* were detected and their associations with growth traits were investigated among Egyptian water buffaloes. According to the published data, this is the first study that showed the association between *MyoD* and *MyoG* and growth traits in Egyptian buffalo.

Knowledge on *MyoD* and *MyoG* gene polymorphisms is limited, and little is known about its effect on growth and muscle development in farm animals. In *MyoG*, most detected SNPs are located in exon 1. A T201G SNP causing synonymous mutation was identified in exon 1 of *MyoG* in water buffaloes (Wang et al., 2011). A similar T201G SNP in exon 1 was also detected in cattle *MyoG*, but unlike in buffalo, this SNP caused alanine aa to proline aa non synonymous mutation (Bhuiyan et al., 2009). In contrast, we did not find this SNP in all examined 200 Egyptian buffalo. Instead, we detected

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two novel SNPs; T1198C at nucleotide number 556 of intron 1 and C2858T at nucleotide number 625 of 3′UTR, among Egyptian buffalos and 43 SNPs compared with Chinese buffalo (GenBank accession EF636460). We also detected 23 SNPs in *MyoD* of Egyptian water buffaloes as compared to Italian buffalo (FJ194946), and 43 SNPs in *MyoG* compared with water buffaloes in China (EF636460). However, validating of these polymorphisms effect should be investigated in both Egyptian and foreign buffaloes in a further study.

Previous studies have studied the effect of some SNPs in exon 1 of the *MyoG* gene on meat production, carcass quality and growth traits in farm animals. A non-synonymous A959G SNP (serine/cysteine) affected meat quality characteristics and was significantly associated with water holding capacity and meat tenderness (Ujan et al., 2011b). In addition, a T314C SNP significantly affected rump length, hucklebone width, waist height and body length (Xue et al., 2011), and a T201G SNP which had a significant effect on live weight in cattle (Bhuiyan et al., 2009). A C489T SNP in exon 1 of pig *MyoD* was detected and showed association with carcass muscling which CC genotype was the most favorable for some traits characterizing carcass muscling (Urbanski et al., 2006). Furthermore, the same SNP was greatly affected muscle fiber characteristics and mRNA expression which could be taken as marker for increasing the meat content of carcass in pig (Lee et al., 2012). On the other hand, some other SNPs were detected but were not associated with meat production, carcass quality and growth traits in farm animals. A non-synonymous C624G SNP (serine/cysteine) did not influence meat quality traits in cattle (Ujan et al., 2011a). a C510A SNP in intron 1 of *MyoD* was not significantly associated with any meat quality traits tested (Liu et al., 2008). We failed to detect any SNPs in exon 1 and so no association analysis was performed on exon 1.

The comparison of growth traits with *MyoD* SNPs (A1481C in intron 2 and C1876T in 3′UTR) showed no statistically significant differences among Egyptian buffaloes from birth to the age of 2 years. These results agree with various previous studies, which found non-significant association between *MyoD* gene SNPs and meat quality and growth traits (Zhang et al., 2007; Liu et al., 2008; Ujan et al., 2011a). In contrast, some SNPs were detected in intron 2 and 3′UTR of *MyoD* and showed a significant association with meat production, carcass quality and growth traits in other animals. In cattle, C39T and C112G SNPs in intron 2 had a high effect on living weight, carcass weight and loin eye area (Tian et al., 2007)In yak, C1976T SNP in 3′UTR of *MyoD* showed significant association with heart girth and body weight (Chu et al., 2012). In duck, a A359T SNP in intron 2 was associated with the traits; leg muscle weight, carcass weight, breast muscle weight, dressing percentage, eviscerated percentage, leg muscle weight percentage and lean meat percentage at 8 weeks of age (Wu et al., 2012).

Our results also reported no significant association between the two SNPs in *MyoG*; T1198C in intron 1 and C2858T in 3′ UTR, with growth traits in Egyptian buffalo. In consistence, a C2417T SNP in the 3′UTR region of *MyoG* was not also associated with growth traits in cattle (Bhuiyan et al., 2009).

Conclusion

Association of detected SNPs in *MyoD* and *MyoG* with average daily gain in Egyptian buffalo at the age from birth to 2 years were studied and they revealed no significant correlation with the studied periods. Therefore, these SNPs couldn't be taken as markers for selection of meat producing Egyptian buffalo.

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Table 1. Forward and reverse primer sequences for four *MyoD* loci and five loci of the *MyoG* gene, annealing temperatures (Ta), size of PCR products (bp), and localization of the representative parts of these loci.

Locus	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)	Ta (°c)	Size (bp)	Localization
MyoD.1	CGTGCTGCTATGCTGCTTAC	TAGTCGTCTTGCGTTTGCG	58	440	Exon 1
MyoD.2	TGTGGGCCTGCAAGGCG	GATGCTGGACAGGCAGTCG	58	1258	Exon 1 – 3
MyoD.3	GAACTGCTACGACCGCAC	CAGAGCACCTGGTAAATC	55	552	Exon 2 – 3
MyoD.4	GCGCTTCTGCTAGCCGACG	CGCCTCTCCTACCTCGGG	60	312	Exon 3 – 3/UTR
MyoG.1	ACGTCTTGATGTGCAGCAAC	CTTCTTGAGTCTGCGCTTCT	58	459	5/UTR – Exon 1
MyoG.2	CTGAGAGAGAAGCGCAGACT	CTGGCTCAACTAGACCGACT	58	681	Exon 1 –Intron 1
MyoG.3	GGTATGGCCAGTAGAGAGGA	TGTTGCAGGAGGCCTAGA	56	699	Intron 1 – Intron2
MyoG.4	TTCTAGGCCTCCTGCAAC	AGAAGTGGTGGCATCTGTG	56	587	Intron2 – 3/UTR
MyoG.5	CCACAGATGCCACCACTT	AGCATCAGTCATCTCCTCC	60	754	3/UTR

Table 2. SNPs detected in *MyoD* of Egyptian buffalo as compared to Italian buffalo (FJ194946).

Position		Changed Nucleotide
Nucleotide	locus	
11	Exon 1	C/T**
402	Exon 1	G/A*
403	Exon 1	C/T**
679	Exon 1	C/T**
133	Intron1	G/T
206	Intron1	T/A
246	Intron1	G/A
322	Intron1	A/G
327	Intron1	C/T
377	Intron1	T/A
409	Intron1	G/T
432	Intron1	C/G
449	Intron1	G/A
63	Intron2	A/G
65	Intron2	T/C
137	Intron2	C/G
149	Intron2	A/G

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160	Intron2	G/T
210	Intron2	A/G
215	Exon3	A/G*
19	3′UTR	C/T
45	3′UTR	A/G
110	3′UTR	T/C

* Synonymous mutation; ** Non- synonymous mutation

Table 3. SNPs detected in MyoG of Egyptian buffalo as compared to Chinese buffalo (EF636460).

Position		Changed Nucleotide
Nucleotide	locus	
90	Exon1	T/C*
105	Exon1	C/T*
141	Exon1	A/C*
162	Exon1	T/G*
171	Exon1	T/C*
309	Exon1	C/T*
343	Exon1	A/C*
466	Exon1	G/C**
62	Intron1	G/A
74	Intron1	C/T
129	Intron1	A/C
159	Intron1	G/A
174	Intron1	G/T
197	Intron1	T/C
343	Intron1	T/C
398	Intron1	T/C
416	Intron1	G/A
427	Intron1	T/C
447	Intron1	G/A
480	Intron1	C/T
493	Intron1	G/T
529	Intron1	C/T
556	Intron1	C/T
594	Intron1	T/G
698	Intron1	C/T
18	Intron2	T/C
42	Intron2	A indel
43	Intron2	C indel
47	Intron2	C/T
58	Intron2	T/C
59	Intron2	G/C
108	Intron2	C/A
120	Intron2	T/G
156	Intron2	T/G
188	Intron2	A/C
194	Intron2	T/C
196	Intron2	T/G
133	3′UTR	C/G
232	3′UTR	C/T
317	3′UTR	A/G
363	3′UTR	T/C
706	3′UTR	G/T
707	3′UTR	C/T

* Synonymous mutation; ** Non- synonymous mutation

Table 4. Genotype distribution and allelic frequencies at A1481C, C1876T SNPs of *MyoD*, and T1198C and C2858T SNPs of *MyoG* and the estimated Chi-Square (χ^2), HWE = Hardy-Weinberg equilibrium.

SNP	Genotype frequencies			Allele frequencies		χ^2 (HWE)	*P* value
A1481C (*MyoD*)	AA	AC	CC	A	C	19.65	> 0.05
	0.245(49)	0.33(66)	0.425(85)	0.41	0.59		
C1876T (*MyoD*)	CC	CT	TT	C	T	15.49	> 0.05
	0.215(43)	0.34(68)	0.445(89)	0.385	0.615		
T1198C (*MyoG*)	TT	CT		T	C	7.22	> 0.05
	0.39(78)	0.61(122)		0.695	0.305		
C2858T (*MyoG*)	CC	TC		C	T	13.43	> 0.05
	0.335(67)	0.665(133)		0.668	0.332		

Table 5. Association of genotypes at *MyoD*.3 locus with growth traits in Egyptian buffalo.

Age	Growth traits	Genotype		
		AA	AC	CC
Birth	BW(Kg)	33.11±0.22	32.80±0.25	32.66±0.21
3Mo	BW(Kg)	85.67 ±0.26	82.11±0.23	81.77 ±0.20
Birth – 3Mo	ADG(Kg)	0.58±0.03	0.55 ±0.01	0.55±0.05
6Mo	BW(Kg)	139.77±0.20	156.21±0.23	150.34±0.24
3 – 6Mo	ADG(Kg)	0.60±0.03	0.82 ±0.01	0.76±0.04
9Mo	BW(Kg)	191.65±0.23	205.40 ±0.22	185.71 ±0.23
6 – 9Mo	ADG(Kg)	0.58±0.02	0.55±0.04	0.39±0.06
12Mo	BW(Kg)	257.54 ±0.21	246.26 ±0.23	243.10 ±0.23
9 – 12Mo	ADG(Kg)	0.73±0.06	0.45±0.07	0.64±0.03
18Mo	BW(Kg)	335.22 ±0.40	321.60 ±0.50	319.90 ±0.20

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12 – 18Mo	ADG(Kg)	0.43±0.06	0.42 ±0.03	0.43±0.05
24Mo	BW(Kg)	418.70 ±2.03	413.55 ±2.41	410.82 ±2.33
18 – 24Mo	ADG(Kg)	0.46±0.04	0.51±0.06	0.50±0.04

BW- bodyweight, ADG - average daily gain.

Values are least squares means (± SEM).

Table 6. Association of genotypes at *MyoD*.4 locus with growth traits in Egyptian buffalo.

Age	Growth traits	Genotype		
		CC	CT	TT
Birth	BW(Kg)	32.69±0.20	32.80±0.24	32.89±0.20
3Mo	BW(Kg)	84.60 ±0.24	83.02±0.22	80.76 ±0.21
Birth – 3Mo	ADG(Kg)	0.58±0.01	0.56 ±0.01	0.53±0.05
6Mo	BW(Kg)	138.01±0.21	155.32±0.22	151.76±0.24
3 – 6Mo	ADG(Kg)	0.59±0.10	0.80 ±0.02	0.79±0.03
9Mo	BW(Kg)	195.21±0.21	204.89 ±0.23	186.56 ±0.20
6 – 9Mo	ADG(Kg)	0.64±0.02	0.55±0.01	0.39±0.05
12Mo	BW(Kg)	249.66 ±0.20	244.33 ±0.22	242.22 ±0.21
9 – 12Mo	ADG(Kg)	0.60±0.05	0.44±0.03	0.62±0.02
18Mo	BW(Kg)	336.20 ±0.30	320.89 ±0.50	319.12 ±0.20
12 – 18Mo	ADG(Kg)	0.48±0.06	0.43 ±0.03	0.43±0.05
24Mo	BW(Kg)	417.50 ±2.00	410.24 ±2.43	405.63 ±2.70
18 – 24Mo	ADG(Kg)	0.45±0.03	0.50±0.02	0.48±0.05

BW- bodyweight, ADG - average daily gain.

Values are least squares means (± SEM).

Table 7. Association of genotypes at *MyoG*.3 locus with growth traits in Egyptian buffalo.

Age	Growth traits	Genotype	
		TT	CT
Birth	BW(Kg)	32.20±0.26	32.55±0.20
3Mo	BW(Kg)	85.40 ±0.23	83.14±0.20
Birth – 3Mo	ADG(Kg)	0.59±0.01	0.56 ±0.02
6Mo	BW(Kg)	154.41±0.25	150.20±0.21
3 – 6Mo	ADG(Kg)	0.77±0.04	0.75 ±0.02
9Mo	BW(Kg)	209.33 ±0.21	200.80±0.20
6 – 9Mo	ADG(Kg)	0.61±0.05	0.56±0.04
12Mo	BW(Kg)	255.50 ±0.24	249.28 ±0.21
9 – 12Mo	ADG(Kg)	0.51±0.06	0.54±0.03
18Mo	BW(Kg)	330.30 ±0.40	328.21 ±0.70
12 – 18Mo	ADG(Kg)	0.42±0.05	0.44 ±0.06
24Mo	BW(Kg)	419.44 ±2.00	416.78 ±2.39
18 – 24Mo	ADG(Kg)	0.50±0.04	0.49±0.09

BW- bodyweight, ADG - average daily gain.

Values are least squares means (± SEM).

Table 8. Association of genotypes at *MyoG*.5 locus with growth traits in Egyptian buffalo.

Age	Growth traits	Genotype	
		CC	TC
Birth	BW(Kg)	31.98±0.20	32.01±0.24
3Mo	BW(Kg)	83.67 ±0.21	86.22±0.20
Birth – 3Mo	ADG(Kg)	0.57±0.03	0.60 ±0.01
6Mo	BW(Kg)	155.11±0.20	150.24±0.21
3 – 6Mo	ADG(Kg)	0.79±0.02	0.71 ±0.01
9Mo	BW(Kg)	211.30 ±0.20	200.85±0.23
6 – 9Mo	ADG(Kg)	0.62±0.03	0.56±0.06
12Mo	BW(Kg)	257.12 ±0.22	248.98 ±0.20
9 – 12Mo	ADG(Kg)	0.51±0.04	0.53±0.02
18Mo	BW(Kg)	331.45 ±0.31	327.22 ±0.50
12 – 18Mo	ADG(Kg)	0.41±0.06	0.43±0.10
24Mo	BW(Kg)	420.00 ±2.10	413.77 ±2.44
18 – 24Mo	ADG(Kg)	0.49±0.06	0.48±0.07

BW- bodyweight, ADG - average daily gain.

Values are least squares means (± SEM).

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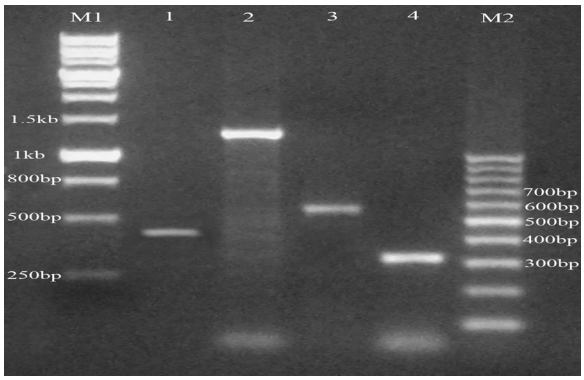

Fig.1. Ethiduim bromide stained agarose gel of PCR products of *MyoD* four loci: lane1 *MyoD.1* (440bp), lane2 *MyoD.2* (1258bp), lane3 *MyoD.3* (552bp), lane4 *MyoD.4* (312bp), M1 lane DNA ladder (1kb) and M2 lane DNA ladder (100bp).

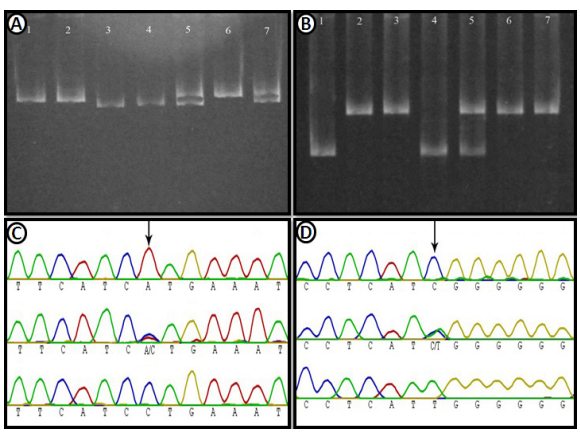

Fig. 2. PCR-SSCP patterns, nucleotide and amino acid sequences of *MyoD*.3 and *MyoD*.4 loci in Egyptian buffalo show the genotypes and polymorphisms. (A) Three SSCP patterns of were detected in *MyoD*.3 locus; genotype CC (lane1, 2 and 6), AA (lanes 3 and 4) and CA (lanes 6 and 7). (B) Three SSCP patterns were determined in *MyoD*.4; genotype CC (lane 1 and 4), TT (lanes 2, 3, 6 and 7) and CT (lanes 5). (C) A A1481C SNP was detected in *MyoD*.3 locus. (D) A C1876T SNP was detected in *MyoD*.4 locus. The arrows indicate the position of SNPs.

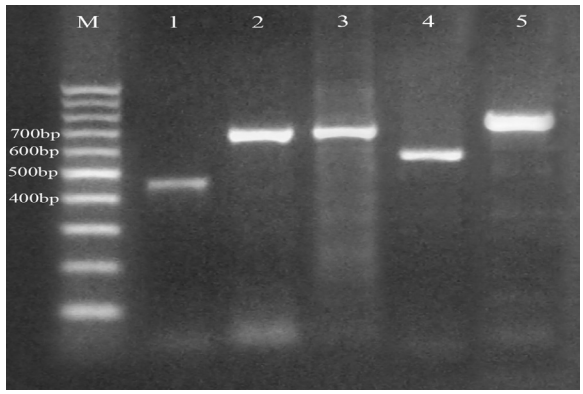

Fig. 3. Ethiduim bromide stained agarose gel of PCR products of *MyoG* five loci: lane1 *MyoG*.1 (459bp), lane2 *MyoG*.2 (681bp), lane3 *MyoG*.3 (699bp), lane4 *MyoG*.4 (587bp), lane5 *MyoG*.5 (754bp) and M lane DNA ladder (100bp).

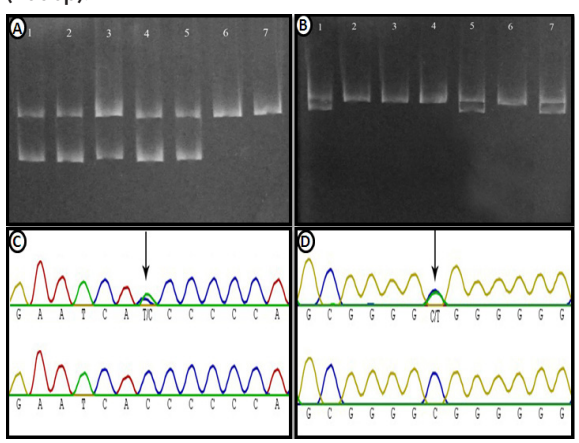

Fig. 4. PCR-SSCP patterns, nucleotide and amino acid sequences of of *MyoG*.3 and *MyoG* loci in Egyptian buffalo show the genotypes and polymorphisms. (A) Two SSCP patterns were detected in *MyoG*.3 locus; genotype TC (lane1-5) and TT (lanes 6 and 7). (B) Two SSCP patterns were determined in *MyoG*.5 locus; genotype CT (lanes 1, 5 and 7) and TT (lanes 2-4 and 6). (C) A T1198C SNP was detected in *MyoG*.3 locus and (D) A C2858T SNP was detected in *MyoG*.5 locus. The arrows indicate the position of SNPs.

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