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Association between polymorphism of Myogenin gene and Growth traits in Cattle and Buffalo

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ABSTRACT

The MyoDfamily of myogenic regulatory factors (MRFs) include several genes such as Myogenin that have the potency to impact economically important traits in beef cattle and therefore it could be used as genetic marker for marker assisted selection (MAS). The current study was aimed at investigating the single nucleotide polymorphism (SNP) inMyogenin gene, estimating the allele and genotype frequencies of the Myogenin gene and to determine the effect of such polymorphism on some growth traits in cattle and buffalo. Genomic DNA was extracted from whole blood of 100 Friesian cattle and 100 Egyptian buffalo. The PCR products of Myogenin gene were subjected to genotyping. PCR-SSCP technique was used to genotype Myogenin gene at the exon-1 and intron-1locus. Two genotypes were determined in cattle(AA and AB). Significant positive association was found between AA genotype and average daily gain. However, no significant association between this genotypeand any other growth traits was detected.Sequencing results revealed a transition of A → G atposition 959 in exon 1 of the MyoG gene in cattle that caused the substitution (959Serine/959Cysteine). These results could be included into marker assisted selection programs to improve the productivity in cattle breed. On the other hand, no polymorphism butonly one genotype was detected in buffalo which is the AB genotype, and accordingly no association analysis was performed in buffalo breed.

Key words: myogenic regulatory factors; Myogenin; genotyping; growth traits; cattle; buffalo

INTRODUCTION

Growth rate is one of the most economically important traits that affect carcass quantity in beef cattle.Animals with higher numbers of muscle fibers give more meat (Rehfeldt et al., 2000). The number of myofibers in muscles is an embryonic process and is regulated by the MyoDgene family (Olson, 1990). The MyoDfamily of myogenic regulatory factors (MRFs) have the ability to transform a variety of cell types into myoblasts (Weintraub et al., 1989). The MyoDfamily of myogenic regulatory factors (MRFs) consists of 4 family members MyF3 (MyoD1), MyF-4or Myogenin (MyoG), MyF-5 and MyF-6genes.

Marker-Assisted Selection (MAS) is a good strategy for genetic improvement of economically important quantitative traits such as growth and carcass traits in beef cattle (Gerbens et al., 2000). Marker-Assisted Selection will first require identification of candidate genes associated with the traits of interest. The candidate gene approach was proposed by many geneticists to identify genes with significant phenotypic performance effects for possible use in genetic improvement programs (Jiang et al., 2002a, b). Candidate genes are selected on the basis of known relationship between physiological or biochemical processes and quantitative traits of interest.

Myogenin is the candidate gene for meat production traits as it has a probable role in muscle fiber development (Verner et al., 2007).

The bovine MyoGgene is located on chromosome 16, along with several QTLs (quantitative trait loci) for carcass weight (Casas et al., 2004),While in buffalo it is located on chromosome 5 (Strazzullo et al., 2010).

MyoGhas an important role within the MyoDgene family, because itsexpression revokes myoblast proliferation potential and regulates the differentiation of mononucleated myoblasts into multinucleated myofibers(Pas and Visscher, 1994), in additionMyoGis the only member of the MyoDgene family that is expressed in all myogenic cell lines (Anton et al., 2002). The MyoGgene regulates the expression of muscle-specific genes, which encode several proteins that control the formation and apoptosis of muscle fibers and some authors suggested that the variation in the number of muscle fibers and growth rate are related to the different MyoGgenotypes (te Pas et al., 1999).Therefore the current study was

aimed at investigating the single nucleotide polymorphism (SNP) inMyogenin gene, estimating the allele and genotype frequencies of the Myogenin gene and to determine the effect of such polymorphism on some growth traits in Friesian cattle and Egyptian buffalo.

Materials and Methods

Sample collection and DNA isolation:

Blood samples were taken from 100 adult Friesian cattle and 100 adult Egyptian buffalo from El WahaElKhadra farm (Cairo-Alex Desert road).The available growth records of the examined animals were obtained from the farm records where they were maintained. The data included birth weight, weaning weight andweightat 6 months. Average daily gain (ADG) for each animal was calculated for the interval between the last two weight records. Genomic DNA was extracted from blood using GF-1 Blood DNA Extraction Kit following the manufacturer protocol. The principle of the kit follows the Spin column-based nucleic acid purification according to**Robert (2008).**

PCR amplification:

One primer pairwas designed based on the bovine MyoGgene sequence (GenBank accession No.EF636458) to amplify exon-1 and intron-1 locus and its flanking regions using the Primer 3.0 software.

Table (1): Primer sequence and information on the bovine MyoGgene exon-1 and intron-1.

Primer sequence	)licon size (bp)	Annealing temperature(°C)
F: GGGGTCCAGAAGCAAGTC R: GGTGAAGGAGGCAGAGTGT	236	58

Table (2):PCR Procedures

1- In a sterile thin walled PCR tube the following components were pipetted:

5X PCR master MIX	5 µl
Forward primer (10 pmol/ µl)	1 µl
Reverse primer (10 pmol/ µl)	1 µl
BSA (2.5 mg/ml)	1 µl
Genomic DNA	X µl (50-100 ng)
Nuclease free water	Up to 25 µl

2-The sample tubes were mixed gently, spun and placed in the thermal cycler (BEOCO Germany) that adjusted to the following program:

Table (3):PCR Program

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	5 minutes	1 cycle
Denaturation	95°C	40 seconds	35 cycles
Annealing	58°C	40 seconds	
Extension	72°C	40 seconds	
Final extension	72°C	10 minutes	1 cycle

The PCR product wasoperated on 1.5% agarose gels (containing 200 ng/ml ethidium bromide) using 1×TAE buffer (0.8 mMTris base, 0.8 mM Glacial acetic acid and 0.02 mM Na_2 EDTA).

PCR-SSCP (single-stranded conformation polymorphism):

Aliquots of 10µL PCR products were mixed with 10 µL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was loaded on a 10% polyacrylamide gel mixture with 99:1 cross-linking rationand electrophoresed at 100 V for 5 h in 1X TBE buffer. The gel was then stained with ethidium bromide solution (1 mg/ml) for few minutes. The individual genotypes were defined according to the PCR-SSCP band patterns that were visualized on the gels with UV light.

Statistical analysis

The following items were statistically analyzed including genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibrium. The association between SNP marker genotypes of the MyoGgene and records of body measurement traits (birth weight, weaning weight,weight at 6 months and Average daily gain) were analyzed byleast square means (LSM±S.E) analysis using the general linear model (GLM) procedure of the statistical analysis system **(SAS/STAT) program, version 7, 2002).**

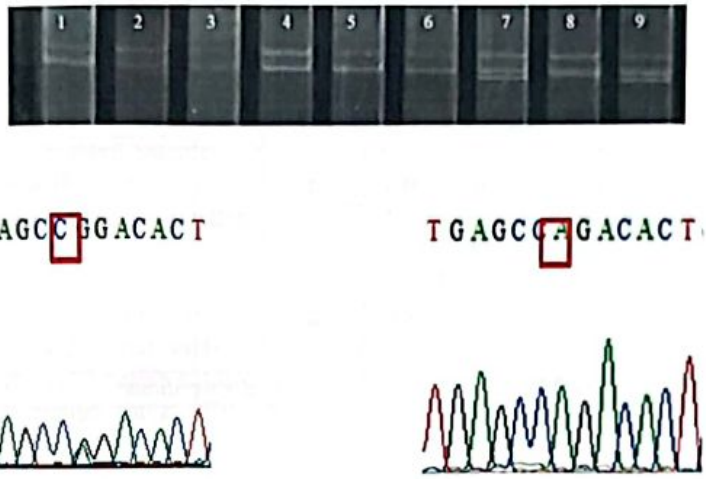
RESULTS

PCR-SSCP analysis of the MyoGgene

After a 236-bp product of the MyoGgene was amplified **(Figure 1),** two unique banding

patterns were detected by PCR-SSCP analysis in cattle. Sequence analysis revealed A>G mutation, a non-synonymous mutation at position 959 of the MyoGgene (**Figure 2**). The homozygote, consistent with the sequence of GenBank accession No.EF636458, was called the AA genotype, and the heterozygote was called the AB genotype. While in buffalo only one banding pattern was detected.

Figure 1. MyoGgene exon 1 PCR amplification products.Lane M = Marker; lanes 1-12 = PCR products.



AB GenotypeAA Genotype

Figure 2.PCR-SSCP patterns and DNA sequencing traces of the cattleMyoGgene locus.Two patterns (AA, AB) were observed (Lanes 1, 2, 3, 4, 5, 6 and 8represent the AA pattern, while lanes 7 and 9 represent the AB pattern. Sequencing trace revealed anA>G mutation at position 959.

Table (4): Genotypes frequencies of myogenin Exon-1 and intron-1 and HWE in Friesian cattle.

myogenin Exon-1 and intron-1 in Friesian cattle (N = 100)					
	Genotypes			Allelic frequency	
	AA	AB	BB	A	B
Observed Number	71	29	0	0.855	0.145
Expected Number	73.1025	24.795	2.1025		
Genotype frequency	0.71	0.29	0.00		
Chi square (X^2).	2.87^{NS}				

Table (5): least-square means and standard errors for growth traits across the Myogenin Exon 1& Intron-1 locus genotypes of Friesian cattle.

Growth Trait	Genotypes			
	AB (n = 29)		AA (n = 71)	
	LSM	±SE	LSM	±SE
BW (kg)	36.54^{a}	0.46	35.28^{a}	0.57
WW (kg)	78.69^{a}	0.69	76.69^{a}	0.79
W6 (kg)	127.80^{a}	0.95	128.79^{a}	1.20
ADG (kg/day)	0.62^{b}	0.007	0.67^{a}	0.012

Values with different subscripts within the row differ significantly at $P < 0.05$.
BW: birth weight, **WW**: weaning weight, **W6**: weight at 6 months and **ADG**: average daily gain, **LSM**: least square means, **SE**: standard errors.

Fig. (3): PCR-SSCP patterns of the buffalo MyoG gene locus. All lanes show the same banding pattern which is the AB genotype.

Table (6): Genotypes frequencies of myoG Exon-1 and Intron-1 and HWE in Egyptian Buffalo.

myoG Exon-1& Intron-1in Egyptian Buffalo (N = 100)					
	Genotypes			Allelic frequency	
	AA	AB	BB	A	B
Observed Number	0	100	0	0.50	0.50
Expected Number	25	50	25		
Genotype frequency	0	100 %	0		
Chi square (X^2).		100	Statistically significant		

DISCUSSION

As a result of MyoGaction in early development and growth of muscle, several studies had investigated the SNPs of this gene and their effect on carcass quality and animal growth. In bovine MyoG, SNPs in exon 1have significant associations with animal growth traits and carcass quality (Bhuiyan et al., 2009; Wang et al., 2011; Xue et al., 2011and Ujan et al., 2011). In pig MyoG, SNP in exon 1 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 and Stupka et al., 2012).Some SNPs are detected in the MyoGgene of deer (Song et al., 2010) and goat (Liu et al., 2011) but their statistical analysis showed no correlation with carcass quality and/or growth traits. These numbers of myogenin SNPs of different phenotypic consequence supports the opinion that further investigation of myogenin SNPs in different breeds is valuable.

Since in MyoG, most detected SNPs are located in exon 1(Wang et al., 2011) we screened this region by using SSCP technique.

Amplification of Exon-1 and Intron-1 of myogenin gene was performed using primer pair developed for cattlebut also succeeded in amplifying the same target sequence in Egyptian buffalo. Fig. (1) showed that the obtained PCR product of the studied breeds appeared as single specific bands with 236bp molecular size.

Identification of genetic polymorphism within this myogenin locus was carried out by SSCP. Separation of denatured PCR products on 10% denaturing PAGE (99:1crosslinking ratio) yielded two banding patterns in Friesian cattle (AA, AB) (Fig. 2) and one banding pattern in Egyptian buffalo (AB) (Fig. 3).

Genotypes and genotypic frequency:

SSCP analysis of the amplified products in Friesian cattle indicated the presence of 2 genotypes; AA and AB (Fig.2). The frequencies of the two genetic variants were 0.71 and 0.29 respectively (table 4) where the differences between observed genotypic frequencies and those expected under HWE were not significant at p-value of 0.05, suggesting that the Friesian cattle population sample was in equilibrium for the Myogenin Exon-1 and Intro1 locus. Only one genotype (AB) was identified among Egyptian buffalo in the present study(Fig.

the other genetic variants in Egyptian buffalo resulted in high degree of deviation from HWE for this gene locus (Table 6).It is difficult to confirm whether the reduced number of genotypes in Egyptian buffalo is a characteristic of this breed or due to the sampling factor, as the samples tested were not chosen to be representative across the Egyptian buffalo breeds.

The nonpolymorphic nature of myoG gene in Egyptian buffalo in our study disagreed with the polymorphism that identified by Wang et al. (2011) and Abu El-Magd et al. (2013). While in Friesian cattle, our results suggested a transition of A → G at position 959 in exon 1 of the MyoG gene, a nonsynonymous mutation that caused the substitution (Serine/Cysteine). This result is in consistence with Ujan et al., (2011) who detected the same mutation in Chinese cattle.

Allelic frequencies:

Two different alleles (A and B) of myoG exon-1 and intron-1 were recorded in our study. The two alleles appeared in both Friesian cattle and Egyptian buffalo. In Friesian cattle, allele A occurred with a frequency of 0.855 while allele B occurred with a frequency of 0.145. On the other hand, both A and B alleles appeared with equal frequency in Egyptian buffalo.

Effect of myogenin genotypes on some growth traits:

There were no significant effects of the recorded genotypes in Friesian cattle myogenin exon-1 and intron-1 on birth weight (BW), weaning weight (WW) and weight at 6 months (W6) **Table (5)**. On the other hand, a significant positive effect on average daily gain was observed in individuals with AA genotype (LSM = **0.67** kg/day) compared to those with AB genotype (LSM = **0.62** kg/day). While in Egyptian buffalo we failed to detect any SNPs in exon 1 and intron-1 and so no association analysis was performed.

Inthis study, the results suggested an association between single nucleotide polymorphisms in the Myogenin gene and growth traits in Friesian cattle (Table 5). This study furnished that the exon-1 959bpA>G mutation was significantlyassociated with average daily gain but at the same time dissimilar results for birth weight, weaning weight and weight at 6 months have been observed. In accordancewith this study,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., 2011)**. In addition, a T314C SNP significantly affected rump length, hucklebone width, waist height and body length **(Xue et al., 2011)**. Several other studies also reported significantassociations with carcass and meat quality traits **(Te Pas et al., 1999; Kapelanski et al., 2005 andVerner et al., 2007)**. On the other hand **Abu El-Magd et al. (2013)** 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 MyoGwas not also associated with growth traits in cattle **(Bhuiyan et al., 2009)**. On the basis of our findings, it is inferred that mutations of MyoG gene can significantly bring about changes in meat characteristics and quality.

CONCLUSION

In this study, the association between single nucleotide epolymorphisms of MyoG gene and growth traits wasstudied in Friesian cattle and Egyptian buffalo. In Friesian cattle, it was found thatthe genotypes were significantly associated withthe average daily gain (ADG), but at the same time dissimilar results forbirth weight (BW), weaning weight (WW) and weight at 6 months (W6) have beenobserved. Our results confirm legion of the previouslyreported significant associations, but diverge with respectto others demonstrating that further investigations arerequired for the effect ongrowth and meat quality traits of geneticvariation in the bovine MyoG gene. It is also suggestedthat this SNP could be used for marker-assisted selection,but a huge number of samples would be required forthis job.

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الملخص العربي

تضم عائلات العوامل المنظمة للعضلات جينات متعددة مثل جين الميوجينين الذى له تأثير عالى على بعض الخصائص الأنتاجيه ذات الأهمية الأقتصاديه و الذى يمكن أستخدامه كدلاله وراثية لإختيار الماشيه بهدف تحسين و زيادة الأنتاجية فيما يسمى بالإختيار المدعوم بالدلالات الوراثية (MAS). تهدف هذه الدراسه إلى التعرف على تعدد اشكال النوكليوتيده الواحده (SNP) فى جين الميوجينين و تقدير معدل تكرار الأليلات و الأنماط الجينيه فى هذا الجين و معرفة تأثير هذه الأنماط الجينيه على بعض خصائص النمو فى الأبقار و الجاموس.تم عزل الحامض النووى الديوكسى ريبوزى من عينات دم من 100 من البقر الفريزيان و 100 من الجاموس المصرى. تم تعريض نواتج ال PCR لجين الميوجينين الى التنميط الجينى. و قد تم استخدام تقنية PCR-SSCP لمعرفة الأنماط الجينيه لجين الميوجينين فى موضع إكسون-1 و إنترون-1. تم تحديد نمطين جينيين فى الأبقار و هما (AA and AB). و وجدت علاقه معنويه بين النمط الجينى AA و متوسط زيادة الوزن اليومى فى حين لا توجد علاقه معنويه بين الأنماط الجينيه و أياً من خصائص النمو الأخرى. كشفت نتائج التسلسل التغيير الذى حدث منA → Gفي موقع 959 في اكسون 1 منجين الميوجين ينفي الأبقار والذى تسبب في استبدال (Serine/Cysteine). ويمكن إدراجه ذهال نتائج إلى برامج الإختيار المدعوم بالدلالات الوراثية لتحسين الأنتاجيه فى هذه السلاله من الأبقار. من ناحيه أخرى لم يتم الكشف عن تعدد الأشكال الجينيه فى الجاموس حيث يوجد نمط جينى واحد وهو AB, لذلك لم يتم إجراء تحليل الأرتباط فى هذه السلاله من الجاموس.