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Association Between Polymorphism Of Myf5 Gene And Growth Traits In Bovine

Els. T. Awad, S. A. Abdelaziz, M. A. Warda and I. M. Ahmad.
Dept. of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Cairo University.

ABSTRACT

Carcass quantity is largely affected by growth rate which is one of the most economically important quantitative traits. MYF5, a member of the myogenic regulatory factors, was chosen as candidate gene for growth traits due to its important role in growth and development of mammals. The objectives of this study were to determine the single nucleotide polymorphism (SNP) in MYF5 gene, estimate the allele and genotype frequencies of the MYF5 gene and to determine the effect of this 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 MYF5 gene were subjected to genotyping. PCR-SSCP technique was used to genotype MYF5 gene at the intron-1 locus. Only one genotype (AB) was detected in cattle and so no association analysis was performed. While in buffalo two genotypes were determined (AC and BB). Significant positive association was found between BB genotype and weight at 6 months and average daily gain. However, no significant association between the genotypes and birth weight and weaning weight was observed. Sequencing results revealed a transition of A → G at position 1385 in intron-1 of the MYF5 gene in buffalo. These results could be included into marker assisted selection programs to improve the productivity in these buffalo breed.

Key words: myogenic regulatory factors; MYF5; genotyping; growth traits; buffalo; cattle.

INTRODUCTION

Egyptians prefer beef to other types of meat including poultry and lamb. The local beef consumption in 2014 was estimated at about 520 thousand metric ton (TMT), up about 4 percent from the 2013 level. Egypt normally imports 40 percent of its beef needs to bridge the gap between domestic production and consumption. Egyptian consumers also prefer fresh over frozen imported beef as they view frozen imported beef as being an inferior product (livestock and product annual, USAD, 2014). So, Improvement in the efficiency of meat production became necessary to meet consumer needs and to face this increasing competition from imported beef products(Miller,2010).

The use of traditional methods of genetic selection which based on phenotypic information has been successful although the nature of genes affecting the economically important traits is not known (Chung and Kim,2005).However, Progress achieved with traditional selection is slow. In addition, some traits cannot be improved very efficiently for reasons such as low heritability of the traits, difficulty or expense in collecting phenotypes, or phenotypes collected later in life (Dekkers,2004). Recent developments in molecular genetic techniques have made it possible to identify genetic variation at specific loci and the association between variation at gene affecting QTL and production traits (Chung and Kim, 2005). The process of determining the differences in the genotype of an individual at a specific DNA region is called genotyping. While the process of selection for a particular trait using genetic markers is called marker assisted selection (Williams, 2005). MAS is a novel technique for selection of individuals to become parents for next generations through the using of molecular markers that can compliments to the traditional selective breeding methods for rapid genetic gains (SaraFarokhzadeh and Barat AliFakheri, 2014).

Myogenic factor 5 (MYF5) was chosen as candidate gene for growth traits due to their important role in growth and development of mammals.MYF5 gene was mapped at bovine chromosome 5 which was identified as having significant associations with the growth traits (Li et al., 2002; 2004). MYF5 is the first factor of the MyoDgene family to be expressed in the embryo (Braun et al., 1989; 1990). Expression of MYF5 in adult skeletal muscle is restricted to the satellite cell population (Beauchamp et al., 2000) and to the muscle spindles (Zammit et al., 2004). MyF-5 gene is considered as a candidate gene for the meat production and quality traits because of its probable role in muscle fiber development (Verner et al., 2007). Therefore the current study was aimed at investigating the single nucleotide polymorphism (SNP) in MYF5 Gene, estimate the allele and

genotype frequencies of the MYF5 gene and to determine the effect of these polymorphism on some growth traits in cattle and buffalo.

MATERIALS AND METHODS

Sample collection and DNA isolation:

Blood samples were taken from100 adult Friesian cattle and 100 adult Egyptian buffalo from El Waha El Khadra 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 and weight at 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 toRobert (2008).

PCR amplification:

One primer pair was designed based on the bovine MYF5gene sequence (GenBank accession No.M95684) to amplify intron-1 locus and its flanking regions using the Primer 3.0 software.

Table(1): Primer sequence and information on the bovine MYF5 gene exon-1 and intron-1.

Primer sequence	Amplicon size (bp)	annealing temperature(°C)
F: GGCCTCCACTGTCCCA R: GCAGTGCTTGCCCACC	401	60

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	60°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(singlestrandedconformation 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 ratioand 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 MYF5gene 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 MYF5gene

After a 401-bp product of the MYF5gene was amplified **(Figure 1),** one banding pattern was detectedby PCR-SSCP analysis in cattle **(Figure 2)** andtwo banding patterns were detected in buffalo. Sequence analysis for the two banding

pattern in buffalo revealed A>G mutationat position 1385bp of the MYF5gene (**Figure3**). The homozygotewas called the BB genotype, and the heterozygote was called the AC genotype.

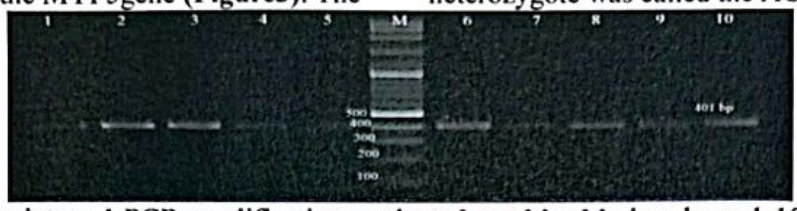


Figure 1.MYF5gene intron-1 PCR amplification products.Lane M = Marker; lanes 1-10= PCR products.

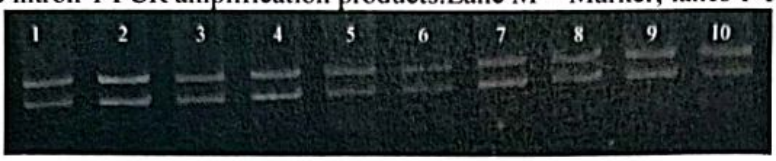
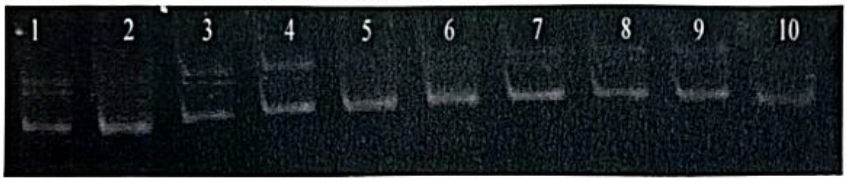
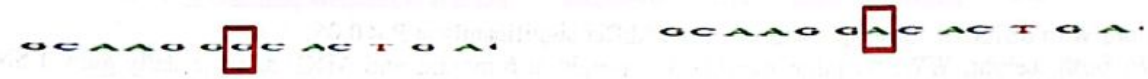


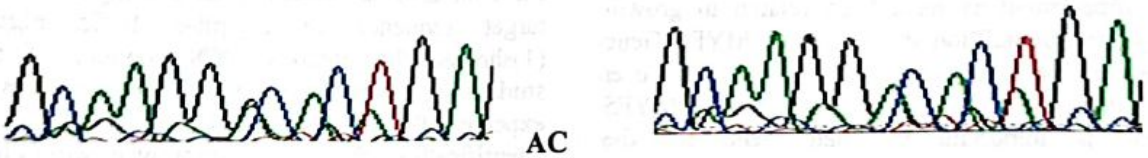
Figure 2: PCR-SSCP patterns of the cattleMYF5gene locus. All lanes show the same banding pattern which is the AB genotype.

Table (4): Genotypes frequencies of MYF5 Gene Intron 1 and HWE in Friesian cattle.

MYF5 Gene Intron 1 in Friesian cattle. (N = 100)						
	Genotypes			Allelic frequency		
	AA	AB	BB	A	B	C
Observed Number	0	100	0	0.50	0.50	0.00
Expected Number	25	50	25			
Genotype frequency	0	100%	0			
Chi square (X^2).	100 statistically significant					







AC

Genotype **BB Genotype**

Figure 3.PCR-SSCP patterns and DNA sequencing traces of the buffaloMYF5gene locus.Two patterns (BB, AC) were observed (Lanes 1, 3, 4, 7, 8 and 9represent the AC pattern, while lanes 2, 5, 6 and 10 represent the BB pattern. Sequencing trace revealed anA>G mutation at position 1385bp.

Table (5): Genotypes frequencies of MYF5 Gene Intron 1 and HWE in Egyptian Buffalo.

MYF5 Gene Intron 1 in Egyptian Buffalo. (N = 100)									
	Genotypes						Allelic frequency		
	AA	AB	BB	AC	CC	BC	A	B	C
Observed Number	0	0	36	64	0	0	0.32	0.36	0.32
Expected Number	10.24	23.04	12.96	20.48	10.24	23.04			
Genotype frequency	0	0	36%	64%	0	0			
Chi square (X^2).	200 statistically significant								

Table (6): LSM and SE for growth traits across the MYF5 Gene Intron 1 locus genotypes of Egyptian Buffalo.

Growth Trait	Genotypes			
	BB (n = 36)		AC (n = 64)	
	LSM	±SE	LSM	±SE
BW (kg)	35.72[a]	0.77	34.29[a]	0.54
WW (kg)	77.42[a]	0.94	77.08[a]	0.75
W6 (kg)	136.39[a]	1.02	129.61[b]	0.79
ADG (kg/day)	0.74[a]	0.003	0.64[b]	0.002

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.

DISCUSSION

In meat producing animals like cattle and pigs, myofiber numbers have been related to growth capacity (Soumillion et al., 1997). MYF5 Gene plays a role in myocyte differentiation (Braun et al., 1989; Li et al., 2004). Therefore, the MYF5 gene is important candidate gene for the identification of genetic markers for growth and carcass traits in livestock. SNPs in bovine MYF5, have significant associations with animal growth traits and carcass quality ((Li et al., 2004; Chungand Kim, 2005; Zhang et al., 2007; Bhuiyan et al., 2009 Ujan et al., 2011a,b,c). SNPs in pig MYF5 is associated with growth rate, and carcass quality (Carmo et al., 2005; Kunhareang et al., 2009). These numbers of MYF5 SNPs of different phenotypic consequence supports the opinion that further investigation of MYF5 SNPs in different breeds is valuable.

Amplification of Intron-1 of MYF5 gene was performed using primer pair developed for cattlebut also succeeded in amplifying the same target sequence in Egyptian buffalo.Figure (1)showed the obtained PCR product of the studied breeds appeared as single specific bands of expected 401bp molecular size.

Identification of genetic polymorphism within this MYF5 locus was carried out by SSCP. Separation of denatured PCR products on 10% denaturing PAGE (99: 1 crosslinking ratio) yieldedone banding patterns in Friesian cattle (AB) Figure (2) and two banding patterns in Egyptian buffalo (BB and AC) Figure (3).

Genotypes and genotypic frequency:

SSCP analysis of the amplified products in Friesian cattle indicated the presence of only one genotype (AB) (Figure 2).The reduced number of genetic variant resulted in high degree of deviation from HWE for this gene locus (table 4).While in Egyptian buffalo2 genotypes were detected;

BBand AC(Figure 3). The frequencies of the two genetic variants were 0.36 and 0.64 respectively (table 5) the differences between observed genotypic frequencies and those expected under HWE were statistically significant at p-value of 0.05, suggesting that the Egyptian buffalo population sample was not in equilibrium for theMYF5 Intro-1 locus.

Up to our knowledge the genetic variation in MYF5 intron-1 has not been previously reported for Egyptian buffalo.

Ujan et al. (2011c) identified a substitution SNP g.1142 A > G in the intron1 region of the MyF-5 Gene in Chinese cattle. Instead, we detecteda novel SNP; A1385G in the intron-1 region of MyF-5 in Egyptian buffalo (Figure 3).

Allelic frequencies:

Three different alleles (A, B and C) of MYF5 Intro-1 were recorded in our study. Allele C appeared only in Egyptian buffalo with a frequency of 0.32. Both A and B alleles appeared with equal frequency in Friesian cattle. On the other hand, allele A occurred with a frequency of 0.32 while allele B occurred with a frequency of 0.36 in Egyptian buffalo.

Effect of MYF5intron-1 genotypes on some growth traits:

We failed to detect any SNPs in intron-1 in Friesian cattle and so no association analysis was performed.While in Egyptian buffalo there were no significant effects of the recorded genotypes on birth weight (BW) and weaning weight (WW). On the other hand, a significant positive effect on weight at 6 months (W6) and on average daily gain was observed in individuals with BB genotype (LSM for W6 = 136.39 kg), (LSM for ADG = 0.74 kg/day) compared to those with AC genotype (LSM for W6 = 129.61 kg), (LSM for ADG = 0.64 kg/day)**Table (6)**.

Inthisstudy,theresultssuggestedanassociationbetwe ensinglenucleotide polymorphisms in the buffaloMYF5 gene and growth traits in Egyptian buffalo (Table 6). This study furnished that the intron-1 1385bpA>G mutation was significantlyassociated with weight at 6 months andaverage daily gain but at the same time dissimilar results for birth weight and weaning weight have been observed. In consistence, previous studies on the MYF5 intron-1 mutations proved that the substitution SNP g.1142 A > G is significantly associated with Intramuscular fat, rib area and water holding capacity (Ujan et al., 2011c).

CONCLUSION

In this study, the association between single nucleotidepolymorphisms of MYF5 gene and some growth traits wasreported in Egyptian buffalo. It was found thatthe genotypes were significantly associated withthe weight at 6 months (W6) andaverage daily gain (ADG), but at the same time dissimilar results forbirth weight (BW) and weaning weight (WW) have beenobserved. Our results confirm legion of the previouslyreported significant associations, but further investigations arerequiredfor the effect ongrowth and meat quality traits of geneticvariation in the buffaloMYF5 gene. It is also suggestedthat this SNP could be used for marker-assisted selection,but a huge number of samples must be requiredforthisjo.

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الملخص العربي

الأرتباط بين تعدد الأشكال الجينيه لجين MYF5 و النمو فى الماشيه

تتأثر كمية الذبيحة بالحد كبير بمعدل النمو والذي يعد واحد من أهم الصفات الكمية من الناحية الاقتصادية تم أختيار MYF5 وهو عضو من جينات العوامل المنظمه للعضلات كجين مرشح لخصائص النمو و ذلك نتيجه لدوره الفعال فى نمو و تطور الثدييات. الهدف من هذه الدراسه هو التعرف على تعدد اشكال النوكليوتيده الواحده (SNP)فى جين MYF5 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 عدد مائه من البقر الفريزيان و مائه من الجاموس المصرى. تم تعريض نواتج ال PCR لجين MYF5 الى التنميط الجينى. تم استخدام تقنية PCR-SSCP لمعرفة الأنماط الجينيه لجين MYF5 فى موضع إنترون-1. تم تحديد نمط جينى واحد فى الأبقار وهو (AB) لذلك لم يتم إجراء تحليل الأرتباط. بينما فى الجاموس تم تحديد نمطين جينيين و هما (AC and BB). وقد وجد إرتباط معنوى بين النمط الجينى BB و الوزن عند ستة أشهر و معدل زيادة الوزن اليومى فى حين لا يوجد إرتباط معنوى بين الأنماط الجينيه و وزن الولاده و وزن الفطام.كشفتنتائجالتسلسلالتغيير الذى حدث من G → A فيموقع1385فياترون 1 منجينMYF5فيالجاموس.ويمكنإدراجهذهالنتائج فى برامج الإختيار المدعوم بالدلالات الوراثية لتحسين الأنتاجيه فى هذه السلاله من الجاموس.