Association of Fatty Acid Binding Protein 4 (FABP4) Polymorphisms with Growth and Carcass Traits of Barki Sheep

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Abstract: Some of the breeding goals for sheep are to increase growth rate and lean meat content and to decrease fat content of carcasses. The measurements of these traits are laborious and expensive by slaughtering animals based on traditional selection method. Therefore, molecular marker can improve selection programs. The objective of this study was to identify allelic and genotype polymorphisms in two regions (exon2-intron2 and exon3-intron3) of fatty acid binding protein 4 (FABP4) gene using polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) tool, and also estimating the association of the detected genotype with growth and carcass traits of Egyptian Barki lambs. In the first region, SSCP analysis showed two alleles (A_1 and A_2 with frequency of 0.74 and 0.26 respectively) and three genotypes (A_1A_1, A_1A_2 and A_2A_2 with frequency of 0.53, 0.43 and 0.04 respectively). In the second region, SSCP analysis showed four alleles (B_1, B_2, B_3 and B_4 with frequency of 0.449, 0.409, 0.089 and 0.044 respectively) and eight genotypes (B_1B_1, B_1B_2, B_1B_3, B_1B_4, B_2B_2, B_2B_3, B_2B_4 and B_3B_3 with frequency of 0.160, 0.430, 0.131, 0.022, 0.137, 0.050, 0.056 and 0.014 respectively). General linear effect models revealed that SSCP genotypes in the first region had significant ($P< 0.05$) effect on Flank% and pH meat. Also revealed that the SSCP genotypes in the second region significantly ($P< 0.05$) associated with marketing weight, tail% and fat%, and high significantly ($P< 0.01$) associated with lean meat%.
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1. Introduction

Growth and carcass traits are economically important traits in meat producing animals. Finding effective molecular markers for these traits has been a challenge in animal genetics for decades. The use of polymorphic specific genes as molecular markers is a promising alternative to the current methods of trait selection, once these genes are proven to be associated with traits of interest in animals (Dario et al., 2009).

Fatty acid-binding proteins (FABPs) are a family of carrier proteins for fatty acids and other lipophilic substances such as eicosanoids and retinoids (Chmurzyńska 2006; Smathers and Peterson 2011). These proteins are thought to facilitate the transfer of fatty acids between extra and intracellular membranes (Weisiger 2002). There are nine subtypes of FABPs exhibit unique patterns of tissues expression and are expressed most abundantly in tissues involved in active lipid metabolism. These subtypes are: adipocyte (A-); brain (B-), epidermal (E-), heart (H-), ileal (Il-), intestinal (I-), liver (L-), myelin (M-) and testis (T-) FABP (Furuhashi and Hotamisligil 2008).

Adipocyte-fatty acid binding protein (A-FABP), also called FABP4, is predominantly produced in adipose tissue and has a crucial role to transport fatty acids in adipocytes of mammals. The main function for this gene is thought to be in lipid metabolism (Storch and Corsico 2008). Studies on FABP4-null mice have demonstrated that it is important in the maintenance of glucose and lipid metabolism (Hotamisligi et al., 1996; Cao et al., 2008). Thus, $FABP4^{-/-}$ mice are protected from development of obesity-induced insulin resisitance and diet-induced atherosclerosis (Makowski et al., 2001; Maeda et al., 2005; Boord et al., 2002; Uysal et al., 2000).

FABP4 is ranging in size from 14-15 kDa containing 128-132 amino acids (Zimmerman and Veerkamp 2002), and encoded by FABP4 gene. This gene has been identified in human, cattle, chicken and pigs, and across these species it has conserved structure with 4 exons separated by 3 introns.

Few studies concerned the variation in FABP4 and its association with economically important traits of meat animals. In cattle, Michal et al. (2006) searched for polymorphisms in bovine FABP4 gene in DNA pools of animals with high and low marbling scores and identified significant association between AAFC_01136716.1:g.7516G>C SNP genotypes and backfat thickness and marbling score in Wagyu×Limousin F_2 population. Also, Cho et al. (2007) revealed that 220A>G (174v) and 348+303T>C polymorphisms in FABP4 showed putative associations with backfat thickness. A SNP

located in exon 3 of FABP4 (g.3691G>A) was associated with increases of marling score and meat quality grade in Hanwoo cattle (Shin et al., 2012). Intramuscular fat content was found to be associated with potential SNP markers in *Bos taurus* FABP4 (Jurie et al., 2007; Wang et al., 2005; Pannier et al., 2010).

Recently, FABP4 polymorphisms were reported that may be linked to chicken body fat content. Wang et al. (2006) detected a C/T substitution in the exon 1 of chicken FABP4 gene that did not change the coding region, but statistical analysis indicated that the substitution was correlated with chicken abdominal fat content. Luo et al. (2006) reported that the same substitution mutation in exon 1 was also significantly correlated with abdominal fat, subcutaneous fat and intramuscular fat content of chicken. A novel G/A polymorphism in exon 3 of the chicken FABP4 gene was associated with abdominal fat percentage (Wang et al., 2009).

A SNP polymorphism in intron 1 of ovine FABP4 was associated ($P< 0.05$) with meat tenderness, muscle marbling score and intramuscular fat content in three Chinese native sheep (Xu et al., 2011).

The aim of this study was to identify variation in two separate regions of ovine FABP4 gene and its association with growth and carcass traits of Egyptian Barki sheep.

2. Materials and Methods

Data Collection

This study was carried out at Maryout Research Station, Desert Research Center. One hundred and thirty seven male and female Barki lambs phenotyped for growth traits (birth weight, pre-weaning average daily gain, weaning weight, post-weaning daily gain and marketing weight).

Blood samples were drawn from the jugular vein into 5 ml heparinized tubes. These samples were stored at – 80 °c for several months, whereupon genomic DNA extracted with the use of DNA extraction kit (Promega).

At marketing age (9 months), 34 male lambs were randomly selected, fasted for 18 hours and then slaughtered by severing the carotid artery and jugular veins. After slaughtering, carcasses were skinned and eviscerated before weighing. Weights of all abdominal and thoracic offal's (trachea, lungs, heart, liver, testes, spleen, kidneys, abdominal fat and kidney fat) were recorded immediately after removal from the body. The rumen and reticulum were cleaned and washed under cold running water, and then they were weighed. Then all carcasses were chilled at an average temperature of 4° C for 24 h to evaluate cold carcass weight.

After chilling, each carcass was divided into seven cuts (Legs, Loins, Racks, Flank, Shoulders, Neck and Tail) according to the Egyptian wholesale mutton cuts as described by Hamada (1976). Chilled carcasses and wholesale cut were weighed to calculate percentages of chilled carcass weight.

The 9-10-11 rib cut was separated into its physical components (lean meat, fat and bone), which were expressed as percentages of the weight of the whole rib cut.

Carcass traits which recorded were: hot carcass weight, chilled carcass weight, dressing % 1, dressing %, neck %, shoulder %, rack %, loin %,, flank %, leg %, tail %, rib (10-11) weight, lean-meat %, fat %, bone %, loss % and pH of meat.

Polymerase chain reaction (PCR) and genotyping

Two sets of specific primers (table 1) were used to amplify region 1 (exon2-intron2) and region 2 (exon3-intron3) of ovine FABP4. The sequences of the first set of primers are described in Burrows (2013). The sequences of the second set of primers based on the published bovine FABP4 DNA sequence (ENSBTAG00000037526) and ovine FABP4 mRNA sequence (NM_001114667).

Table (1). The sequence of two set of specific primers

	Primer sequence (5`-3`)
Region 1	F1: CAGGAATTTGATGAAGTCACT
	R1: GTAACATGGTTCAGAGCTAG
Region 2	F2: GATGGGAAATCAACCACCA
	R2: TCTCCTTCAATGCTGAGAAG

PCR carried out in a total reaction volume of 20 ul, containing 2.5 ul of 10x PCR buffer. 1.5 mM of MgCl2, 150 *u*M of dNTP (Eppendorf, Hamburg, Germany), 0.25 uM of each primer, 50 ng of genomic DNA and 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany). The thermal profile consisted of 2 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 60°C and 30s at 72°C, with a final extension of 5 min at 72°C.

PCR products were subject to single strand conformational polymorphism (SSCP) analysis in 14% polyacrylamide gels at 320 V and 12 °C for 18 hours in 0.5X TBE buffer and gels were silver-stained using the method of Sanguinette at al. (1994).

Statistical analysis

The association of different genotypes with phenotypic of the studied traits was evaluated by two general linear models analysis using SAS software (SAS 1989). The first model was used to test the association between SSCP genotypes and growth traits. However, the association between SSCP genotypes and carcass traits was tested using the

second model. Due to the low frequency of B_1B_4 and B_3B_3 genotypes, the statistical analysis was performed without them.

Statistical model I: $Y_{ijkl} = \mu + R1_i + R2_j + S_k + e_{ijkl}$

Statistical model II: $Y_{ijk} = \mu + R1_i + R2_j + e_{ijk}$

Where Y= trait value; μ = general mean; $R1$ = the fixed effect of SSCP genotype in region 1; $R2$ = the fixed effect of SSCP genotype in region 2; S = the fixed effect of sex of animal and e = random residual.

3. Results and Discussion

Allelic and genotype frequencies

PCR-SSCP analysis of the two amplified regions of FABP4 is shown in table 2, table 3 and figure 1. In the first region, only two alleles (A_1 and A_2) and three genotypes (A_1A_1, A_1A_2 and A_2A_2) were identified. However, four alleles (B_1, B_2, B_3 and B_4) with eight genotypes (B_1B_1, B_1B_2, B_1B_3, B_1B_4, B_2B_2, B_2B_3, B_2B_4 and B_3B_3) were identified in the second region.

Table (2). Allelic frequencies for two regions of FABP4 gene

Region / Allele	Region 1		Region 2			
	A1	**A2**	**B1**	**B2**	**B3**	**B4**
Observed frequency	0.74	0.26	0.449	0.409	0.098	0.044

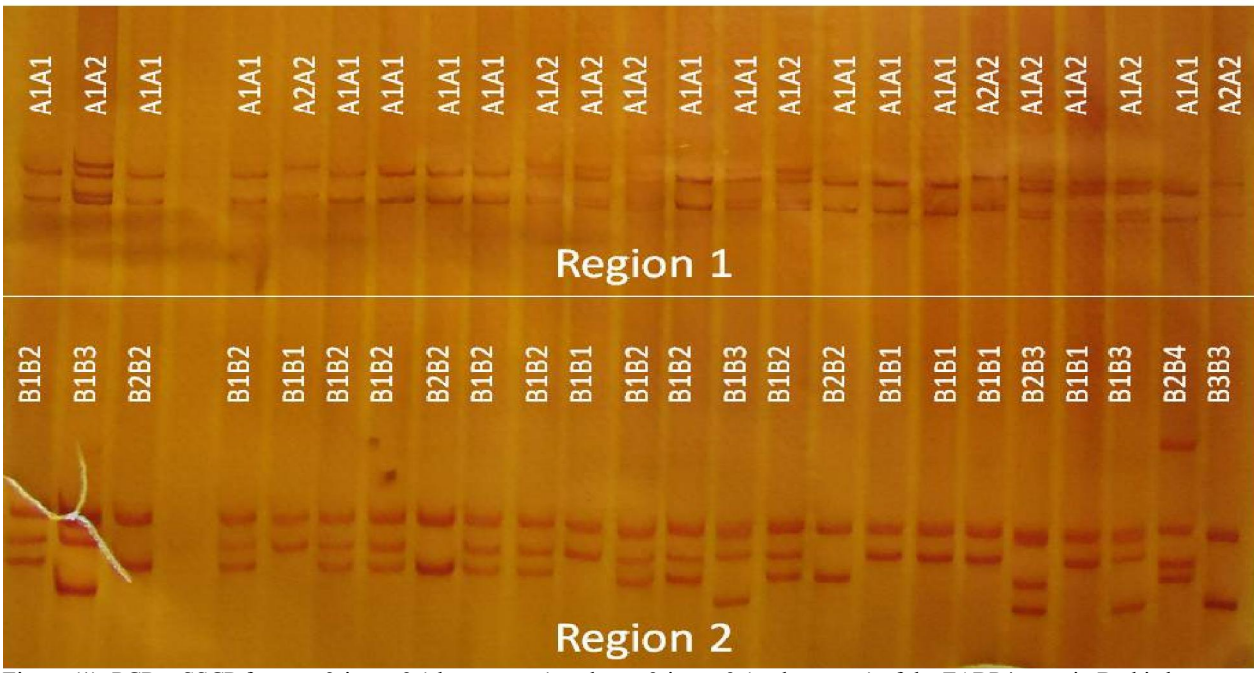

Figure (1). PCR – SSCP for exon2-intron2 (above arrow) and exon3-intron3 (under arrow) of the FABP4 gene in Barki sheep.

Chi-square test based on observed and expected frequencies of different SSCP genotypes in the two regions of FABP4 gene showed significant deviation from Hardy-Weinberg equilibrium. The obtained results showed that the FABP4 gene had a high degree of polymorphisms and could be considered as a candidate gene causing variation in the phenotypic values of growth and carcass traits of Barki sheep.

Table (3). Genotype frequencies for two regions of FABP4 gene.

Region	**Region 1**			**Region 2**							
Genotype	**A_1A_1**	**A_1A_2**	**A_2A_2**	**B_1B_1**	**B_1B_2**	**B_1B_3**	**B_1B_4**	**B_2B_2**	**B_2B_3**	**B_2B_4**	**B_3B_3**
Animal number	73	59	5	22	58	18	3	19	7	9	1
Observed frequency	0.53	0.43	0.04	0.16	0.430	0.131	0.022	0.137	0.050	0.056	0.014

Association between sex and the studied traits

Sex of animal high significantly ($P< 0.01$) affected post weaning average daily gain and have no effect on the other traits.

Association between FABP4 genotypes and the studied traits

The effect of SSCP genotypes in region 1 and region 2 of FABP4 gene on growth and carcass traits are presented in tables 4 and 5 respectively. The

SSCP genotypes in region 1 have only significant effect (P< 0.05) on flank % and meat pH, and did not have any significant effect on phenotypic values of the other studied traits. Lambs with heterozygous genotype A_1A_2 had higher flank % than lambs with homozygous genotypes A_1A_1 and A_2A_2. This result - demonstrates additive genetic effect for those two alleles on flank %.

Table (4). The effect of SSCP genotypes in region 1 of FABP4 gene on phenotypic values of the studied traits

Trait	Mean ± Std Error			P-value
	A_1A_1	A_1A_2	A_2A_2	
Growth traits				
Birth weight (KG)	3.94±0.07	3.57±0.08	3.58±0.08	0.818
Weaning weight (Kg)	19.55±0.42	19.57±0.49	20.58±1.86	0.956
Marketing weight (Kg)	41.93±0.87	43.27±1.20	39.75±3.42	0.134
ADG1 (gm/d)	173.6±4.05	174.0±4.76	185.4±18.96	0.986
ADG2 (gm/d)	82.18±2.73	87.14±3.35	70.12±11.99	0.059
Carcass traits				
Hot carcass weight (Kg)	18.86±0.60	18.91±0.62	19.11±1.85	0.324
Dressing 1%	45.16±0.47	44.22±0.59	48.42±4.16	0.165
Dressing2%	55.57±0.50	54.86±0.97	61.25±7.94	0.270
Neck%	7.24±0.19	7.08±0.22	7.48±0.99	0.182
Shoulder%	19.93±0.38	19.59±0.22	20.47±0.94	0.066
Rack%	25.25±0.24	24.09±0.29	24.40±0.71	0.087
Loin%	6.50±0.25	6.62±0.31	6.36±0.64	0.965
Flank%	**3.88±0.17**	**4.19±0.16**	**3.40±0.23**	**0.013***
Leg%	34.11±0.23	34.53±0.47	35.15±1.12	0.295
Tail%	3.08±0.21	3.15±0.23	2.72±0.34	0.124
9-10-11 rib weight (gm)	541±32.4	579±33.5	635±70.9	0.192
Lean meat %	48.24±1.76	47.94±1.45	42.32±1.74	0.145
Fat%	17.47±1.08	20.17±1.34	25.96±1.58	0.900
Bone%	30.56±0.98	29.94±0.62	29.87±1.48	0.403
Loss%	2.65±0.51	1.92±0.22	1.84±0.62	0.380
pH	**6.19±0.25**	**5.69±0.06**	**6.62±1.11**	**0.011***

Significance level * refers to significance at (P < 0.05) and ** refers to significance at (P < 0.01)

In the second region of FABP4 gene, SSCP genotypes significantly (P< 0.05) affected marketing weight, and high significantly (P< 0.01) affected lean meat %. The average phenotypic value of B_1B_1 lambs for marketing weight and lean meat % were significantly (P< 0.05) higher than the B_1B_3 lambs. Marketing weight and lean meat ratio are very important in sheep industry because of their high economic values. For this reason, they have been improved dramatically in the past decades through different methods, such as marker assisted selection. These traits are complex traits that involve an increase in body mass and maturation of many tissues especially for skeletal muscles. In the present study, the ovine FABP4 gene that was chosen as a candidate gene to evaluate its SSCP with the aim of investigating the genetic association of FABP4-SSCP with growth and carcass traits approved positive effect on those two important traits and we suggest that genotype B_1B_1 could be regarded as a molecular marker for superior marketing weight and lean meat %.

The results also showed significant effect (P< 0.05) for SSCP genotypes in region 2 on tail % and fat%. The B_1B_3 heterozygous had higher tail % and higher fat % (P< 0.05) compared to B_1B_1 homozygous. The association results between SSCP genotypes of FABP4 gene and fat content of carcasses are consistent with previous investigations on other meat animals. These investigations have been focused on the detection of polymorphisms in FABP4 gene and revealed significant associations for the detected polymorphisms with intramuscular fat of sheep (Xu et al., 2011); backfat thickness and intramuscular fat of cattle (Michal et al., 2006; Cho et al., 2007; Jurie et al., 2007; Wang et al.,2005; Pannier et al., 2010); and also with body and abdominal fat contents of chicken (Wang et al., 2006; Luo et al., 2006; Wang et al., 2009). Excessive fat depositions in lambs should be limited to enhance production efficiency and product quality. Similar to human, pig

and chicken FABP4 genes, the ovine FABP4 is expressed only in fat tissues, in which it is extremely abundant. Our results indicated to increase fat deposition in tails and carcasses of lambs that carrying allele B_3; therefore, it would be possible to make selection against this allele to get carcasses with small tail and less fat content.

Table (5). The effect of SSCP genotypes in region 2 of FABP4 gene on phenotypic values of the studied traits

Trait	Mean ± Std Error						P-value
	B_1B_1	B_1B_2	B_1B_3	B_2B_2	B_2B_3	B_2B_4	
Growth traits							
Birth weight (KG)	3.54±0.13	3.48±0.08	3.53±0.14	3.51±0.13	3.71±0.15	3.72±0.15	0.939
Weaning weight (Kg)	20.75±0.66	19.65±0.52	17.92±0.89	19.39±0.82	20.57±0.99	18.67±1.18	0.243
Marketing weight (Kg)	**48.52±1.74**	**42.67±1.04**	**36.71±1.78**	**42.51±1.74**	**43.28±2.28**	**39.93±2.81**	**0.015***
ADG1 (gm/d)	183.8±5.85	174.1±5.18	160.9±8.84	172.7±7.73	184.4±9.54	162.1±9.99	0.257
ADG2 (gm/d)	90.6±5.81	84.3±2.89	69.7±5.89	85.2±5.62	102.1±4.93	78.3±7.96	0.058
Carcass traits							
Hot carcass weight (Kg)	21.12±1.62	18.61 ±0.06	16.75±0.67	19.48±0.93	-	17.55±1.13	0.061
Dressing 1%	44.93±1.20	44.45±0.34	45.69±1.95	45.80±0.57	-	43.90±0.81	0.792
Dressing2%	56.51±1.14	55.22±0.62	56.36±3.36	55.99±0.63	-	54.05±1.03	0.931
Neck%	7.22±0.54	7.11±0.26	4.47±0.36	7.35±0.27	-	7.27±0.37	0.867
Shoulder%	19.60±0.24	19.94±0.38	20.15±0.55	18.75±0.28	-	19.58±0.51	0.076
Rack%	25.05±0.99	24.44±0.31	24.25±0.44	25.11±0.45	-	25.16±0.65	0.890
Loin%	5.90±0.34	6.54±0.24	6.93±0.51	6.64±0.46	-	6.24±0.81	0.833
Flank%	3.77±0.25	3.96±0.21	4.14±0.30	3.99±0.14	-	4.15±0.44	0.213
Leg%	32.96±1.07	34.60±0.42	34.54±0.67	34.64±0.18	-	34.40±0.35	0.363
Tail%	**3.02±0.38**	**3.32±0.25**	**3.52±0.24**	**2.50±0.31**	**-**	**3.21±0.62**	**0.049***
9-10-11 rib weight (gm)	640.0±86.0	539.0±32.6	534.0±37.2	663.0±65.4	-	481±39.9	0.052
Lean meat %	**53.77±1.81**	**47.38±1.13**	**41.91±1.08**	**51.62±2.87**	**-**	**46.75±3.60**	**0.010****
Fat%	**17.48±1.52**	**18.5±1.02**	**24.50±1.13**	**16.18±1.79**	**-**	**18.16±2.30**	**0.021***
Bone%	27.08±0.87	30.58±1.23	31.77±0.94	30.26±1.33	-	30.51±1.22	0.289
Loss%	1.66±0.61	2.79±0.72	1.79±0.23	2.04±0.52	-	2.08±0.37	0.769
pH	5.94±0.29	6.06±0.24	5.95±0.41	5.75±0.11	-	5.70±0.03	0.175

Significance level * refers to significance at ($P < 0.05$) and ** refers to significance at ($P < 0.01$)

In conclusion, it may be stated that FABP4 is a polymorphic gene. The genotype B_1B_1 positively affected marketing weight and lean meat %, however, the genotype B_1B_3 negatively affected tail % and fat % of carcasses. Hence the first genotype may be favored and the second genotype may not be favored in the farm to get carcasses with high lean meat % and less fat %.

References

1. Boord J B, Maeda K, Makowski L, Babaev V R, Fazio S, Linton M F, Hotamisligil G S (2002). Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arteriosclerosis, Thrombosis and Vascular Biology, 22:1686-91.*
2. Burrows L E R (2013). Investigation of the molecular genetic basis of fly-strike resistance in NZ sheep: Analysis of the FABP4 gene. *B. Sc. Dissertation submitted to Lincoln University, Lincoln, Canterbury, New Zealand.*
3. Cao H, Gerhold K, Mayers J R, Wiest M M, Watkins S M, Hotamisligil G S (2008). Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell 134: 933–944.*
4. Chmurzyńska A (2006). The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *Journal of Applied Genetics, 47: 39-48.*
5. Cho S, Park T S, Yoon D H, Cheong H S, Namgoong S, Park B L, Lee H W, Han C S, Kim E M, Cheng Il-C, Kim H, Shin H D (2007). Identification of genetic polymorphism in FABP3 and FABP4 and putative association with backfat thickness in Korean native cattle. *BMB Report 41:29-34.*
6. Dario, C, Selvaggi, M, Carnicella, D, Bufano G (2009). STAT5A/*Ava*I polymorphism in Podolica bulls and its effect on growth performances traits. *Livestock Science, 123: 83-87.*
7. Furuhashi M, Hotamisligil G S (2008). Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nature Reviews Drug Discovery, 7: 489–503.*
8. Hamada, M K O (1976). Mutton and wool production, *Dar El-Maaref, Alexandria, Egypt (in Arabic). 9, 389–396.*

9. Hotamisligil G S, Johnson R S, Distel R J, Ellis R, Papaioannou V E, Spiegelman B M (1996). Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 274: 1377-1379.
10. Jurie C, Cassar-Malek I, Bonnet M, Leroux C, Bauchart D, Boulesteix P, Pethick D W, Hocquette J. F. (2007). Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. *Journal of Animal Science, 85: 2660-2669*.
11. Luo G, Chen J, Wen J, Zhao G, Zheng M, Sun S (2006). Study of single nucleotide polymorphism of A-FABP gene and its association with fatness traits in chickens. *Yi Chuan (China), 28:39–42.*
12. Maeda K, Cao H, Kono K, Gorgun C Z, Furuhashi M, Uysal K T, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn B B, Parker R A, Hotamisligil G S (2005). Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metabolism, 1: 107–119.*
13. Makowski L , Boord J B, Maeda K, Babaev V R, Uysal K T, Morgan M A, Parker R A, Suttles J, Fazio S, Hotamisligil G S, Linton M F (2001). Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nature Medicine, 7: 699-705.*
14. Michal, J J, Zhang Z W, Gaskins C T, Jiang Z (2006). The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. *Animal Genetics, 37:400-402.*
15. Pannier L, Mullen A M, Hamill R M, Stapleton P C, Sweeney T (2010). Association analysis of single nucleotide polymorphisms in DGAT1, TG and FABP4 genes and intramuscular fat in crossbred Bos taurus cattle. *Meat Science, 85: 515-518.*
16. Sanguinetti C J, Dias Neto E, Simpson A J G (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques, 17: 915-919.*
17. SAS (1989). SAS/ STAT, User's guide. Version 6, 4th edition. Volume 2. SAS Institute Inc., Cary, NC, USA.
18. Shin S C , Heo J P, Chung E R (2012). Genetic variants of the FABP4 gene are associated with marbling scores and meat quality grades in Hanwoo (Korean cattle). *Molecular Biology Reports, 39: 5323-5330.*
19. Smathers R L, Petersen D R (2011). The human fatty acid-binding protein family: evolutionary divergences and functions. *Hum Genomics* 5: 170–191.
20. Storch J, Corsico B (2008). The Emerging Functions and Mechanisms of Mammalian Fatty Acid-Binding Proteins. *Annual Review of Nutrition* 28: 73-95.
21. Uysal K T, Scheja L, Wiesbrock S M, Bonner-Weir S, Hotamisligil G S (2000). Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology, 141:3388-3396.*
22. Wang Q, Li H, Li N, Leng L, Wang Y, Tang Z (2006). Identification of single nucleotide polymorphism of adipocyte fatty acid-binding protein gene and its association with fatness traits in the chicken. *Poultry Science, 85:429–434.*
23. Wang Q, Guan T, Li H, Bernlohr D A (2009). A Novel Polymorphism in the Chicken Adipocyte Fatty Acid- Binding Protein Gene (*FABP4*) That Alters Ligand-Binding and Correlates with Fatness. *Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology, 154: 298–302.*
24. Wang Y H, Byrne K A, Reverter A, Harper G S, Taniguchi M, McWilliam S M, Mannen H, Oyama K, Lehnert S A (2005). Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. *Mammalian Genome, 16:201–210*.
25. Weisiger R A (2002). "Cytosolic fatty acid binding proteins catalyze two distinct steps in intracellular transport of their ligands". *Molecular and Cellular Biochemistry,* 239: 35–43.
26. Xu Q L, Tang G W, Zhang Q L, Huang Y K, Liu Y X, Quan K, Zhu K Y, Zhang C X (2011). The *FABP4* gene polymorphism is associated with meat tenderness in three Chinese native sheep breeds. *Czech Journal of Animal Science, 56: 1–6*.
27. Zimmerman A W, Veerkamp J H (2002). New insights into the structure and function of fatty acid-binding proteins. *Cellular and Molecular Life Sciences, 59: 1096– 1116.*