

Evaluation of SCD and FASN Gene Expression in Baluchi, Iran-Black, and Arman Sheep

Mohammad Salmani Izadi¹, Abbas Ali Naserian*², Mohammad Reza Nasiri²,
Reza Majidzadeh Heravi², Reza Valizadeh²

Abstract

Background: With the increasing concern for health and nutrition, dietary fat has attracted considerable attention. The composition of fatty acids in the diet is important because they are associated with major diseases including cancers, diabetes, and cardiovascular disease. The fatty acid synthase (FASN) and stearoyl-CoA desaturase (delta-9-desaturase) (SCD) genes affect fatty acid composition (1). The expression of SCD and FASN genes is related to an increase in conjugated linoleic acid (CLA) in dairy products, which benefits human health. The aim of current study was to investigate expression changes of SCD and FASN genes that resulted from crossbreeding the local Baluchi sheep with alien breeds.

Methods: We collected tissue samples from the mammary glands of 24 single-born ewes from local Baluchi and synthetic Iran-Black and Arman sheep breeds in the Abbas Abad breeding center. After RNA extraction and cDNA synthesis, real-time PCR was performed with all samples in triplicate.

Results: The maximum and minimum expression of SCD and FASN genes was in the local Baluchi sheep and the crossbred Arman sheep, respectively.

Conclusions: With the highest SCD and FASN gene expression in local Baluchi sheep and relatively less expression of these genes in synthetic Iran-Black and Arman Sheep breeds, it may be necessary to consider the consequences of crossbreeding local sheep and the fatty acid composition of their dairy products.

Keywords: SCD, FASN, Gene expression, Real-time PCR

Introduction

There is growing consumer recognition of the link between diet and health. This awareness impacts food choices and the term “functional food” is a generic one often used to describe this concept (1). The functional role of conjugated linoleic acid (CLA) in health has been reviewed by Benjamin and Friedrich (2). Conjugated linoleic acid is a generic term for a range of positional and geometric isomers of linoleic acid that benefit human health. Cis-9, trans-11 CLA is responsible for the anti-carcinogenic properties of CLA, although the mechanisms are still under

study (3). Trans-12, cis-10 CLA decreases fat mass in animals (4). Dairy products provide approximately 75% of human CLA dietary intake. The cis-9, trans-11 CLA in milk fat is the major isomer and represents 78–89% of the total CLA in sheep milk fat (5).

Sheep milk fat contains several components that may benefit human health, such as monounsaturated fatty acids (FAs) and CLA. Most of the CLA in ruminant milk is synthesized in the mammary gland by the action of the enzyme stearoyl-CoA desaturase

1: Department of Animal Sciences, Ferdowsi University of Mashhad, International Campus, Mashhad, Iran.

2: Department of Animal Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.

*Corresponding authors: Abbas Ali Naserian; Tel: +98 9151163228; Fax: +98 5138807141; E-mail: abasalin@yahoo.com

Received: Feb 27, 2016; Accepted: Apr 5, 2016

(SCD) on circulating vaccenic acid (trans-11 C18:2; VA) (6).

The fatty acid synthase (FAS) enzyme is encoded by the FASN gene. Fatty acid synthase catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH into long-chain saturated FAs (7).

The Baluchi sheep is the most common native breed in the RazaviKhorasan, Sistan and Baluchistan, and Kerman and Yazd provinces of the Islamic Republic of Iran, comprising about 30% of the total sheep population, or approximately 15 million heads (8). The Baluchi sheep breed originated in what is now southwest Pakistan, eastern Iran, and southern Afghanistan, also known as Mengali, Kermani, Naeini, Neini, Yazdi, Araghi, and Khorasani, based on geographic distribution. This breed is well-adapted to a wide range of harsh environments from the northeast to the southeast of the country and commonly reared on low-quality pastures via household extensive systems. The small stature and particular physiological characteristics of Baluchi sheep have enabled them to tolerate unfavorable natural conditions. For this reason, Baluchi sheep are categorized as a high-quality mutton-producing breed with low cost. The breed is fat-tailed with white fleece spots of black markings on their heads and legs. They are primarily employed in meat production with wool suitable for carpets (9, 10). This breed plays an important role in the economy of ranchers and meets protein and dairy needs of society (11).

To increase production in this breed, a crossbreeding program was begun in 1975 with Chios sheep, a breed from the Greek island of Chios, in 1975. The breeding project commenced in the sheep-breeding station of Abbas Abad, located in KhorasanRazavi province in northeast Iran. The result of this breeding project was named Iran-Black (12).

Iran-Black was the first synthetic sheep breed in Iran, developed to increase litter size and improve weaning performance, and tolerate the harsh environmental conditions prevalent in the Baluchi sheep region (12). The milk yield of lactating Iran-Black ewes averages 962 grams per day over a 90-day period (13).

The Arman sheep breed was developed in 1976 by crossbreeding Chios, Suffolk, Ghezel, and Baluchi sheep. This breed was developed for arid regions and is well-adapted to the wide range of harsh environmental conditions found in northeastern Iran (13). The crossbred Arman ewes produce more offspring and milk than the local breed ewes (13). The milk yield of lactating Arman ewes averages 958 grams per day over a 90-day period (13).

Crossbreeding offers two primary advantages over the use of only one breed; crossbred animals 1) exhibit heterosis (hybrid vigor), and 2) combine the strengths of the breeds used to form the cross. The goal of a well-designed, systematic crossbreeding program is to simultaneously optimize these advantages (14). Crossbreeding between sheep breeds is common worldwide; however, to our knowledge, no studies of gene expression changes resulting from crossbreeding in sheep have been reported in Iran.

In lactating ruminants, mammary glands have high levels of SCD mRNA (15, 16) and activity (17-22). The SCD protein has a key role in the synthesis of milk monounsaturated FAs and specific CLA isomers by introducing a cis double bond between carbons 9 and 10 of the FAs (23). In milk, about 60% of oleic (cis-9-18:1; a major milk FA), 50–56% of palmitoleic (cis-9-16:1), and 90% of myristoleic (cis-9-14:1) acids, and > 60 % of the major isomer of CLA are synthesized in mammary glands by the action of the SCD enzyme (23). This action is important due to the impact of milk fat concentration and FA profile in determining milk nutritional quality and human health (23).

Indeed, certain saturated (mainly 12:0, 14:0, and 16:0) and trans FAs are considered to exert negative effects when consumed in excess, whereas others (4:0, anteiso-15:0, cis-9-18:1, 18:3 n -3, and some CLA isomers) have potentially positive effects on human health (24, 25). For example, cis-9, trans-11-18:2, the major isomer of CLA in ruminant milk, exhibits anti-carcinogenic and anti-atherogenic properties in animal models (26). Thus, by contributing to the synthesis of FAs that benefit human nutrition, e.g., cis-9, trans-11-CLA, and to a lesser extent cis-9-18:1, SCD improves the nutrition of milk

fat. In addition, by introducing a cis-9 double bond to FAs, SCD decreases the milk fat melting point (24). Differences in SCD protein levels in mammary glands may help to explain the substantial variation in the levels of these FAs in milk fat; thus SCD gene expression in the mammary gland has been of major interest in recent decades.

Another enzyme related to the increase of CLA in dairy products and its beneficial effects on human health is FAS (29). Fatty acid synthase is part of a multifunctional enzyme complex that catalyzes the synthesis of saturated FAs, including myristate, palmitate, and stearate (27- 28).

Analysis of SCD and FASN gene expression on local Baluchi and synthetic breeds are important to understand the unsaturated FA contents of milk. Our hypothesis is that comparing SCD and FASN gene expression in these local and synthetic sheep breeds could offer the effect of crossbreeding on the fat composition of sheep milk.

Materials and Methods

Experimental site and sheep breed

The experiment was conducted at Abbas Abad breeding center of Mashhad in RazaviKhorasan Province during the spring of 2014. Eight each of Baluchi, Arman, and Iran-Black ewes were selected. All were 3 years old and single-born. All the sheep in this study were reared in the same conditions and had ad libitum access to the same ration of grassland diets and water.

Mammary gland biopsy

In late lactation of the ewes, mammary gland tissue was collected with a biopsy needle. The biopsy site was cleaned with 10% povidone-iodine solution, and 2.5 ml of lidocaine hydrochloride was injected subcutaneously. A 3 cm incision was made approximately 2 cm above the nipple to facilitate insertion of the biopsy needle. After the biopsy, the mammary gland surface was sprayed with Aludemin to protect the wound from dirt. Immediately after biopsy, the mammary tissue sample was stored in RNAlater solution (QIAGEN) and then frozen and stored at -80°C .

RNA extraction and cDNA synthesis

Total RNA was prepared from the mammary gland biopsy with TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's instructions. The quantity and quality of the RNA were determined by agarose gel electrophoresis (0.8%, w/v). To exclude possible amplification of contaminating genomic DNA, samples were treated with DNase. Single-stranded cDNA was synthesized from 1 μg of RNA with the RevertaidcDNA synthesis kit (Termofisher Scientific Litvania) following the manufacturer's recommendations.

Real-time polymerase chain reaction analysis (Real-time PCR)

The expression pattern of SCD and FASN genes in these sheep was analyzed by real-time PCR. To normalize the results of the target genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene in all samples. Glyceraldehyde-3-phosphate dehydrogenase was previously reported to exhibit low variation and no significant differences in gene expression levels between adipose tissues in different sheep breeds (30-32).

Gene sequences for forward and reverse primers for SCD, FASN, and GAPDH were obtained from a study by Dervishi et al., 2012 (33) (Table 1). Real-time-PCR was performed using a Bio-Rad CFX96 real-time PCR system (USA). Standard curves for genes were generated to calculate the amplification efficiency. The efficiency of PCR amplification for each gene was calculated with the standard curve method ($E = 10^{-1/\text{slope} - 1}$). The standard curves for each gene were generated by 4-fold serial dilution of pooled cDNA with 1/10, 1/100, 1/1000, and 1/10000 dilutions. The amplification conditions were an initial step of 10 min at 95°C , followed by 40 cycles of 95°C for 15 sec and 59 or 60°C for 30 sec. The specificity of the amplification products and the lack of primer dimers were confirmed by melting curve analysis in all cases. To quantify the relative gene expression, the standard curve method was used according to the recommendation of

Larionov *et al.* (34). Normalized real-time PCR data were transformed to the fold-change relative

to the control group. Expression of Baluchi sheep breed considered as control group.

Table 1. Sequences of primers used in this study.

Primername		Primer sequence 5' →3'	Amplicon (bp)	GenBank accession numbers	Annealing temperature(°C)
SCD	F	atgttgaccacatccccatt	115	AJ001048	57
SCD	R	cccagctgtcagagaaaagg		AJ001048	
FASN	F	gtgtggtacagcccctcaag	110	GQ150557	57
FASN	R	gtgtggtacagcccctcaag		GQ150557	
GAPDH	F	atgctctctgaccacca	76	HM043737	57

Results

Real-time PCR data analysis

The minimum average of cycle threshold for SCD, FASN, and GAPDH genes obtained 24, 25/78, and 23/11, respectively. Figure 1 represents normalized data for SCD and FASN results. The SCD and FASN gene expression in the mammary glands of Baluchi sheep breed was greater than in

the synthetic Iran-Black or Arman sheep. The FASN gene expression was four-fold and two-fold less in the synthetic Arman and Iran-Black than in the Baluchi sheep. The SCD gene expression was 7.5 and 3.5 times less in synthetic Arman and Iran-Black than in Baluchi sheep, respectively (Table 2).

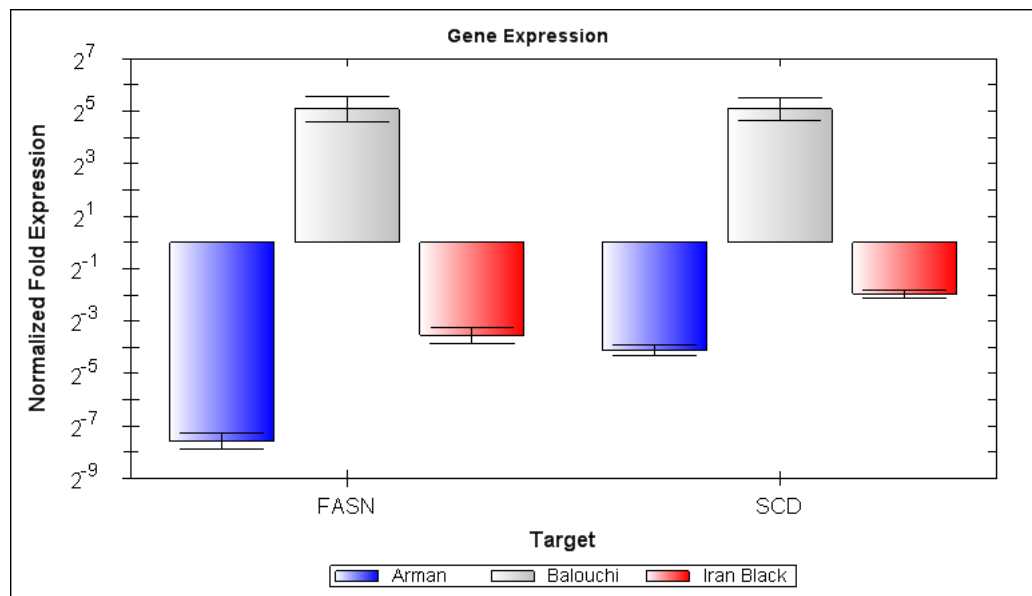


Fig. 1. Fold expression for SCD and FASN genes in Baluchi, Iran Black and Arman breeds.

Table 2. Dietary Intake among the patients with low vitamin D status, and Control Groups^a

Gene	Baluchi	Iran-Black	Arman
SCD	5.08 ± 0.1238	-3.53 ± 0.029	-4.11 ± 0.008
FASN	5.08 ± 0.1429	-1.95 ± 0.021	-7.58 ± 0.001

Discussion

Bakhtiarzadeh et al. (35) studied SCD and FASN gene expression in local Zel lambs and Lori–Bakhtiari sheep; however, they sampled from the fat-tail and visceral adipose tissues and observed relative expression levels of 5.92 and 11.67 for SCD and FASN genes in the Zel lambs and 0.86 and 1.52 in the Lori–Bakhtiari sheep, respectively. But no study in Iran has as yet been reported that evaluated gene expression changes of SCD and FASN in mammary glands of local and synthetic sheep breeds.

Chen et al. (36) found no significant relationship between FASN gene expression and intramuscular fat (IMF) contents of pigs; however, Guo et al. (37) reported a positive relationship between FASN expression and fat deposits in Xiang pigs. Qiao et al. (31) observed a negative relationship between FASN mRNA expression and IMF content in male Kazak sheep. Cui et al. (38) observed no correlation between the FASN mRNA expression and IMF content in chicken breasts and thighs.

Similar to our findings, Dervishi et al. (33) reported relative expression of SCD and FASN under 2-fold in semitendinous muscle of Rasa Aragonesa lambs.

Gene expression as a result of crossbreeding on different cow populations was studied by Paape et al. (39). They observed less gene expression in crossbred cows than in native cow populations. Also Huang et al. (40) reported that crossbreeding can reduce the expression of some genes. In Iran, Moridi et al. (41) found less IL8RB gene expression in the offspring of crossbred

cows than in the native Guilan and Holstein cows used for the crossbreeding.

These studies agree with our results related to decreased gene expression following crossbreeding.

Comparison of the expression of SCD and FASN genes between these local and synthetic sheep breeds is useful in understanding the effect of crossbreeding on FA composition of dairy products.

Until now, no studies have reported that the changes in mammary gland expression of lipid metabolism-related genes can be affected by crossbreeding. The difference we observed may be attributed to a relatively lower contribution of the Baluchi sheep genome than those of the synthetic Iran-Black and Arman sheep.

With consideration of the relatively high SCD and FASN expression in local Baluchi sheep being reduced in the synthetic Iran-Black and Arman Sheep cross-breeds, it may be necessary to consider the effects of crossbreeding on the FA composition of dairy products.

Acknowledgement

This study was supported by Ferdowsi University of Mashhad (Mashhad, Iran). The authors express their appreciation to Mr. M. Jafari (Manager of Animal breeding Center of North East, Iran) and Mr. M. Farshchian (Molecular Medicine Research Department, ACECR-Khorasan Razavi Branch, Mashhad, Iran) for their cooperation.

References

1. Milner JA. Functional foods and health promotion. *J. Nutr.* 1999; 129(7):1395S–97S.
2. Benjamin S, Spener F. Conjugated linoleic acids as functional food: an insight into their health benefits. *NutrMetab (Lond)*. 2009; 6(1): 36–47.
3. Larsson SC, Bergkvist L, Wolk A. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. *Am J ClinNutr.* 2005; 82(4):894–900.
4. Parodi PW. Milk fat in human nutrition. *THE AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY*. 2004; 59(1): 3–59.
5. Luna P, Fontecha J, Juárez M & de la Fuente MA. Conjugated linoleic acid in ewe milk fat. *J Dairy Res.* 2005; 72(4):415–24.
6. García-Fernández M, Gutiérrez-Gil B, García-Gómez E, Sánchez JP, Arranz JJ. Detection of quantitative trait loci affecting the milk fatty acid profile on sheep chromosome 22: role of the stearyl-

- CoA desaturase gene in Spanish Churra sheep. *J Dairy Sci.* 2010; 93(1):348-57.
7. Leonard AE, Pereira SL, Sprecher H, Huang YS. Elongation of long-chain fatty acids. *Progress in Lipid Research.* 2004; 43(1): 36–54.
 8. Gholibeikifard A, Aminafshar M, HosseinpourMashhadi M. Polymorphism of IGF-I and ADRB3 Genes and Their Association with Growth Traits in the Iranian BaluchiSheep. *J. Agr. Sci. Tech.* 2013; 15(1): 1153-1162.
 9. Abbasi, M. A., Abdollahi-Arpanahi, R., Maghsoudi, A., Vaez-Torshizi, R. and Nejati-Javaremi, A. Evaluation of Models for Estimation of Genetic Parameters and Maternal Effects for Early Growth Traits of Iranian Baluchi Sheep. *Small Ruminant Res.* 2011; 104(1): 62-69.
 10. HosseinpourMashhadi, M., EftekhariShahroudi, F. and Valizadeh, R. Estimation of Inherited Parameters for Weight Traits in Baluchi Sheep. *J. Agric. Sci. Nat. Resour.* 2005; 12(1): 77-82.
 11. Tahmoorespur M, Sheikhloo M. Pedigree analysis of the closed nucleus of Iranian Baluchi sheep. *Small Ruminant Research.* 2011; 99(1):1-6.
 12. Rashidi, A. Genetic parameter estimates of body weight traits in Iran-Black sheep. *J. Livest. Sci. Technol.* 2012; 1 (1), 54–60.
 13. LotfiFarokhad M , Roshanfekar H ,Amiri S , Mohammadi K , MirzadehKh .Genetic trend estimation for some of the growth traits in Arman sheep. *J Anim Vet Adv.* 2011; 10(1):1801 – 1803.
 14. Suzuki M, Van Vleck LD. Heritability and repeatability for milk production traits of Japanese Holsteins from an animal model. *Journal of Dairy Science.* 1994; 77(1): 583-588.
 15. Ward RJ, Travers MT, Richards SE, Vernon RG, Salter AM, Buttery PJ, Barber MC. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. *BiochimBiophysActa.* 1998; 1391(2):145–156.
 16. Bernard L, Leroux C, Bonnet M, Rouel J, Martin P, Chilliard Y. Expression and nutritional regulation of lipogenic genes in mammary gland and adipose tissues of lactating goats. *J Dairy Res.* 2005; 72(2):250–255.
 17. Bickerstaffe R, Annison EF. The desaturase activity of goat and sow mammary tissue. *Comp Biochem Physiol.* 1970; 35:653–665.
 18. Kinsella JE. Stearic acid metabolism by mammary cells. *J Dairy Sci.* 1970; 53(12):1757–1765.
 19. McDonald TM, Kinsella JE. Stearyl-CoA desaturase of bovine mammary microsomes. *Arch BiochemBiophys.* 1973; 156(1):223–231.
 20. Bernard L, Rouel J, Leroux C, Ferlay A, Faulconnier Y, Legrand P, Chilliard Y. Mammary lipid metabolism and milk fatty acid secretion in alpine goats fed vegetable lipids. *J Dairy Sci.* 2005; 88(4):1478–1489.
 21. Bernard L, Bonnet M, Leroux C, Shingfield KJ, Chilliard Y. Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in Alpine goats fed maize silage-based diets. *J Dairy Sci.* 2009; 92(12):6083–6094.
 22. Bernard L, Leroux C, Faulconnier Y, Durand D, Shingfield KJ, Chilliard Y. Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. *J Dairy Res.* 2009; 76(1) 241–248.
 23. Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. *Am J PhysiolEndocrinolMetab.* 2009; 297(1):E28-37.
 24. Parodi PW. Dairy product consumption and the risk of breast cancer. *J Am CollNutr.* 2005; 24(6 Suppl):556S–568S.
 25. Shingfield KJ, Chilliard Y, Toivonen V, Kairenius P, Givens DI. Trans fatty acids and bioactive lipids in ruminant milk. *AdvExp Med Biol.* 2008; 606(1):3–65.
 26. Wahle KWJ, Heys SD, Rotondo D. Conjugated linoleic acids: are they beneficial or detrimental to health. *Prog Lipid Res.* 2004; 43(6):553–587.
 27. Smith S, Witkowski A, Joshi A. Structural and functional organization of animal fatty acid synthase. *Progr Lipid Res.* 2003; 42:289-317.
 28. Roy R, Ordovas L, Zaragoza P, Romero A, Moreno C, Altarriba J, Rodellar C. Association of polymorphisms in the bovine FASN gene with milk-fat content. *Anim. Genet.* 2006; 37:215-218.
 29. Han LQ, Pang K, Li HJ, Zhu SB, Wang LF, Wang YB. et al. Conjugated linoleic acid-induced milk fat reduction associated with depressed expression of lipogenic genes in lactating Holstein mammary glands. *Genet. Mol. Res.* 2012; 11 (4): 4754-4764.
 30. Chen, X.J., Mao, H.L., Ma, X.M., Liu, J.X. Effects of dietary corn oil and vitamin E supplementation on fatty acid profiles and expression of acetyl CoA carboxylase and stearoyl-CoA desaturase gene in Hu sheep. *Anim. Sci. J.* 2010; 81, 165–171.

31. Qiao Y, Huang Z, Li Q, Liu Z, Hao C, Shi G. et al. Developmental changes of the FAS and HSL mRNA expression and their effects on the content of intramuscular fat in Kazak and Xinjiang sheep. *J. Genet. Genomics.* 2007; 34, 909–917.
32. Zhang JQ, Smith B, Langdon MM, Messimer HL, Sun GY, Cox RH. Changes in LPL and reverse cholesterol transport variables during 24-h postexercise period. *Am J Physiol Endocrinol Metab.* 2002; 283(2):E267-74.
33. Dervishi E, Serrano C, Joy M, Serrano M, Rodellar C, Calvo JH. The effect of feeding system in the expression of genes related with fat metabolism in semitendinous muscle in sheep. *Meat Sci.* 2011; 89:91–97.
34. Larionov A, Krause A, Miller W. A standard curve based method for relative real time PCR data processing. *BMC Bioinformatics.* 2005; 21:6-62.
35. Bakhtiarizadeh MR, Moradi-Shahrbabak M, Ebrahimie E. Underlying functional genomics of fat deposition in adipose tissue. *Gene.* 2013; 521(1):122–8.
36. Chen J, Yang XJ, Tong H, Zhao RQ. Expressions of FAS and HSL mRNA in longissimusdorsi muscle and their relation to intramuscular fat contents in pig. *J. Agric. Biotechnol.* 2004; 12, 422.
37. Guo W, Wang SH, Cao HJ, Xu K, Zhang J, Du ZL. Gene Microarray Analysis for Porcine Adipose Tissue: Comparison of Gene Expression between Chinese Xiang Pig and Large White. *Asian Australas. J. Anim. Sci.* 2008; 21(1): 11-18.
38. Cui HX, Zheng MQ, Liu RR, Zhao GP, Chen JL, et al. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. *Mol. Biol. Rep.* 2012; 39, 3479–3484.
39. Paape MJ, Shafer-Weaver K, Capuco A. V, VanOostveldt K., Burvenich C. Immune surveillance of mammary tissue by phagocytic cells. *Advances in Experimental Medicine and Biology.* 2000; 480: 259–277.
40. Huang W, Nadeem A, Zhang B, Babar M, Soller M, Khatib H. Characterization and comparison of the leukocyte transcriptomes of three cattle breeds. *PLoS ONE.* 2012; 7: 30244-30251.
41. Moridi M, HosseiniMoghaddam SH, Mirhosseini SZ. Study on IL8RB gene expression in crossbreeding program of Guilan Native cow. *Animal Production Research,* 2015;4(1):25-34.