aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2016.847.853

Research Article
STAT5B Gene Polymorphisms are Associated with Egg Production and Egg Quality Traits in Laying Hens

[1,2]Rangsun Charoensook, [1]Nithat Wichasit, [1]Thitima Pechrkong, [1]Tossaporn Incharoen and [1]Sonthaya Numthuam

[1]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, 65000 Phitsanulok, Thailand
[2]Center for Agricultural Biotechnology, Naresuan University, 65000 Phitsanulok, Thailand

Abstract

Objective: To study the effect of Signal Transducers and Activators of Transcriptions 5B *(STAT5B)* gene polymorphism on egg production and egg quality traits in White Leghorn (WLH) and Rhode Island Red (RIR) chickens. **Methodology:** A total of 80 blood samples were collected and extraction. The PCR-RFLP of *STAT5B* gene was performed by using *MSPI* enzyme to detect a SNP g.4533815G>A. Two fragments (477 and 77) for GG, three fragment (554, 477 and 77) for AG genotype and undigested fragment (554) for AA genotype were identified. **Results:** The genotype frequencies of AA, AG and GG were 0.351, 0.108 and 0.541 in WLH and 0.525, 0.400 and 0.075 in RIR, respectively. Statistical analysis showed that *STAT5B* genotypes were significantly associated with BW of initial ($p<0.01$), feed intake ($p<0.05$), egg weight ($p<0.01$), egg height ($p<0.01$), shell weight ($p<0.01$), shell thickness ($p<0.001$), albumen weight ($p<0.01$) and yolk color ($p<0.001$).The GG genotype has better egg quality traits than both AA and AG genotype. However, there were non-significant difference in rate of lay, egg mass, feed per egg ratio, egg width, shell strength, albumen height, yolk weight and Haugh unit. **Conclusion:** The present study indicated that polymorphisms within *STAT5B* gene should be used as genetic markers in selection programs for improving egg quality trait.

Key words: Egg production, egg quality, laying hen, polymorphisms, *STAT5B* gene

Received: August 03, 2016 Accepted: September 09, 2016 Published: November 15, 2016

Citation: Rangsun Charoensook, Nithat Wichasit, Thitima Pechrkong, Tossaporn Incharoen and Sonthaya Numthuam, 2016. *STAT5B* gene polymorphisms are associated with egg production and egg quality traits in laying hens. Asian J. Anim. Vet. Adv., 11: 847-853.

Corresponding Author: Rangsun Charoensook, Department of Agricultural science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, 65000 Phitsanulok, Thailand

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egg production in Thailand primarily uses commercial breed chickens imported from foreign countries. Though these chickens have good production performance, this type of breed is not suite to the hot and humid weather in Thailand, where the temperature is around 29°C all year. In summer, the temperature may be as high as 40°C with a relative humidity as high as[1,2] 70%. This climate can easily cause hens to experience heat stress which has many negative effects such as a decrease in feed intake and production performance as well as an increase in the mortality rate[3]. Therefore, farmers must renovate their animal housing to incorporate an evaporative cooling system that can control the temperature as well as use high-quality commercial feed. By so doing, farmers will have higher investment costs which present a challenge to small-scale farmers in rural areas who want to produce eggs in this condition. One of the ways to solve this problem and which seems to be a suitable choice is the selection and improvement of chickens. Cross breeding is performed with the aim of appropriate genetic blending to render the offspring fit for a given environment and to induce a heterosis[4]. This can be done by crossbreeding indigenous chickens with selected and still robust exotic breeds in order to have a balance between suitable production performance and tolerance to the environment[4,5]. White Leghorn and Rhode Island Red chickens are pure breed laying hens famous for breed improvement. However, little information has been found about the genetic background of these two breeds in Thailand[2].

Nowadays, molecular genetics techniques are being developed in order to be used as genetic markers in animal breeding. Called Marker Assisted Selection (MAS), this helps in advancing animal breed improvement to reach the target quickly. However, egg production and egg quality traits are controlled by polygenes. As a result, it might be difficult to study the whole genome and the relationship of each gene in egg production. Currently, the popular strategy is to study and compare egg production by using the data in biology, physiology, biochemistry and others. Then, determine the protein or factor that affects the trait of interest to the giving study and after that analyze how gene polymorphism relates to that protein or that factor. This technique is called the direct candidate gene approach. Signal Transducers and Activators of Transcription 5B (STAT5B) have their functions and roles in growth, reproduction, lactation and metabolism[6]. The STAT5B is an important modulator of signal transduction pathway related to growth hormone mechanisms such as the growth hormone receptor, insulin-like growth factor (IGF) and prolactin[7,8]. Moreover, it was found that polymorphisms of the *STAT5B* gene in chickens are associated with growth, body weight on the first day and weight of the first egg[8,9].

According to the previous study of the genetic diversity of the *STAT5B* gene in local chicken population of Thailand[10], this study shows that the Single Nucleotide Polymorphism (SNP) g.4533815G>A can be used as a genetic marker. However, nowadays, studies assessing how such a gene affects the traits in egg production and egg quality are still few and unclear. Therefore, the objective of this study was to study the relationship of the *STAT5B* gene with egg production and eggs quality traits in Rhode Island Red and White Leghorn breeds in the tropical environment of Thailand.

MATERIALS AND METHODS

Experimental population: A total of 80, 31 weeks old Rhode Island Red (RIR, n = 40) and White Leghorn (WLH, n = 40) laying hens from the experimental farm of Naresuan University were used for the experiment. These chickens were raised in cages with separated feed trays and egg collecting trays, each cage contained one chicken and all were fed by limited nutrition according to the percentage of their weights. On average, the chickens were fed twice a day at 7:30 am and 3:30 pm the composition and calculated analyses of the experimental diets are shown in Table 1 and water was fed *ad libitum* by nipple. All birds were exposed to 16 h of light

Table 1: Composition and calculated analyses of the experimental diet

Items	Percentage (%)
Ingredients	
Soybean meal (44% CP)	19.70
Fish meal (58% CP)	5.50
Broken rice (7.6% CP)	11.80
Corn (8% CP)	50.00
Palm oil	2.50
Di-calcium phosphate	2.00
Calcium carbonate	8.00
Salt	0.10
Premix[1]	0.30
DL-methionine	0.10
Calculated analyses	
Crude protein	16.81
Calcium	3.96
Crude fiber	2.62
Lysine	0.90
Meth+Cys	0.67
Available phosphorus	0.52
ME (Mcal kg^{-1})	2.85

[1]Supplied 100 kg^{-1} of diet, vitamin A: 15,000,000 IU, vitamin D: 3,000,000 IU, vitamin E: 26,000 IU, vitamin K: 35 g, vitamin B1: 2.5 g, vitamin B2: 6.5 g, vitamin B6: 257.5 g, vitamin B12: 26 mg, pantothenic acid: 11.04 g, nicotinic acid: 35 g, folic acid: 1.2 g, biotin: 15.1 mg, choline chloride: 250 g, copper: 1.6 g, manganese: 60.2 g, iron: 1.6 g, zinc: 45 g, iodine: 400 mg and selenium: 160 mg

per day. The parameters of egg production performance traits were recorded daily at the same time for 16 weeks. All animal procedures were approved by the Naresuan University Animal Care and Use Committee (NUACUC).

Data collection and traits measured: Data concerning the traits for the rate of lay were analyzed from calculations resulting from the following equations:

$$\text{Rate of lay (\%)} = \frac{\text{No. of eggs}}{\text{No. of existing chickens}} \times 100$$

$$\text{Average egg weight (g)} = \frac{\text{Total weight of all eggs}}{\text{No. of eggs}}$$

$$\text{Egg mass (g HD}^{-1}\text{)} = \text{Average egg production (\%)} \times \text{Average egg weight}$$

$$\text{Feed intake (g day}^{-1}\text{)} = \frac{\text{Total feed consumption}}{\text{No. of feeding days}}$$

$$\text{Feed per egg ratio} = \frac{\text{Total feed consumption}}{\text{Egg mass}}$$

The egg quality data were tested by collecting the data in the 2nd, 4th, 6th, 8th, 10th and 12th weeks of the experiment. The data for egg height, egg width, egg and shell thickness were analyzed by using the digital vernier caliper (Mitutoyo). Egg shape index was calculated from the following formula:

$$\text{Shape index (\%)} = \frac{\text{Egg height}}{\text{Egg width}} \times 100$$

Shell strength was analyzed by using the texture analyzer, series QTS (Brookfield). Yolk color and albumen height were analyzed by using the egg multi-tester, series EMT-7300 (Robotmation Co. Ltd., Tokyo, Japan). The Haugh Unit (HU) scores were calculated for individual egg by using Haugh's equation[11] is as follow:

$$HU = 100\log_{10}(H\text{-}1.7 \times EW^{0.37} + 7.6)$$

where, H is the observed height of the albumen in millimeter and EW is weight of the egg in gram.

Blood sampling and DNA extraction: This process was done by collecting a sample of the chicken's blood from wing vein of all chickens. From each chicken 1-2 mL of blood were taken by using a 3 mL syringe. After that, the blood sample was put into a 5 mL test tube laminated with 0.5 M ethylene-diamine-tetraacetic acid (EDTA). The genomic DNA was extracted from the blood samples with a modified salting out method according to Charoensook[5] and Sambrook *et al.*[12]. Then, the DNA quality and concentration were checked by using the Colibri microvolume spectrometer (Nanodrop®) and diluted to have a concentration of 50 ng μL^{-1} for the Polymerase Chain Reaction (PCR) process.

Genotyping by PCR-RFLP assay: Polymorphism of the *STAT5B* gene was identified by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method adapted from Ou *et al.*[13] and Niknafs *et al.*[14]. The process began with a PCR reaction to amplify DNA fragments consisting of genomic DNA with a 50 ng μL^{-1} concentration; a volume of 2 µL 5X FIREPol® Master Mix Ready to Load (Solis BioDyne); a volume of 4 µL of primer (Forward: 5'- CCA TCC CTT CCT GGT GCA GT -3' and reverse: 5'- ACT GCT GCC ATT TCC CTT TG- 3') with a concentration of 10 µM and a volume of 1 µL. Then, the volume was adjusted to reach 20 µL with ddH_2O. After that, it was taken to the PCR T100TM machine which has 30-cycle reactions, for a 95°C denaturation for 30 sec. Then, it was taken for annealing at 60°C for 30 sec, for extension at 72°C for 1 min, with a 95°C initial denaturation for 3 min and for final extension at 70°C for 5 min. After the reaction process was completed, the PCR products were checked by using 1.5% agarose gel electrophoresis.

The amplified PCR products were digested with fast digest *MSPI* restriction enzyme (Thermo Scientific) for 15 µL per reaction. This consists of 10X fast digest buffer for 1 µL, fast digest enzyme for 0.5 µL, PCR product for 5 µL which is then adjusted to reach 15 µL with ddH_2O. After that, it was incubated at 37°C for atleast 10 min. The genotype patterns were explored by 3% agarose gel electrophoresis. A photo of the genotype pattern was recorded by the gel documentation system (Vilber Lourmat) in order to check the genotype category of each chicken before conducting the statistical analyses in the next step.

Statistical analyses: According to the results of the genotype categorization, the population's genetic structures such as genotype frequency and allele frequency were calculated. The expected heterozygosity (H_e) and the observed heterozygosity (H_o) must be analyzed in order to evaluate the genetic diversity[15] and the Hardy-Weinberg equilibrium was tested using a Chi-square test with the significant level understood as 0.01 and the degree of freedom as 1 with GENALEX Version[16] 6.5.

The association of the *STAT5B* gene with egg production and egg quality traits were analyzed by using the General Linear Model (GLM), the least squares mean was tested by Duncan's multiple range tests by using the SPSS program[17] which has its statistic model as follows:

$$y_{iJ} = \mu + B_i + G_j + e_{ij}$$

where, y_{ij} is the phenotypic record of the observed traits, μ is the overall population mean, B_i is the fixed effect of breeds, G_i is the fixed effect of *STAT5B* genotypes and e_{ij} is the residual error.

RESULTS AND DISCUSSION

Genotype and allele frequencies: The genotype patterns of the *STAT5B* gene in WLH and RIR were checked by using the PCR-RFLP technique by digesting with the fast digest *MSPI* restriction enzyme at the position of SNP g.4533815G>A. According to the study, there were three genotype patterns: AA, AG and GG genotypes (Fig. 1). For the AA genotype, there were undigested DNA fragments, the biggest of which was 554 bp. For the AG genotype, there were DNA fragments at sizes of 554, 477 and 77 bp. Also, for the GG genotype, there were DNA fragments at sizes of 477 and 77 bp. The results of the allele and genotype frequencies of the *STAT5B* gene in WLH and RIR demonstrate that WLH had more frequency of the GG genotype (Table 2) which was also greater than the AA and AG genotypes (0.541, 0.351 and 0.108, respectively). In RIR, it was shown that the frequency of the AA genotype was higher than the AG and GG genotypes (0.525, 0.400 and 0.075, chronologically). According to the result of Chi-square test, it was found that the expansion of the genotype was shown according to the Hardy-Weinberg equilibrium ($p<0.01$), except in WLH which went along with the results of expected heterozygosity (H_e) being higher than observed heterozygosity (H_o). This has shown a low genetic diversity in the population which might result from the cause of selection[10].

Genotype-trait associations: The Signal Transducers and Activators of Transcription 5B (STAT5B) are important modulators for growth hormones, growth hormone receptors, IGF and prolactin which have interrelated roles and which affect the growth and the reproduction of poultry[7-9,18,19]. Thus, it has been suggested that STAT5B may be a candidate gene for growth and egg production traits. According to this study, it was found that the *STAT5B* genotype is associated with the traits of body weight at 31 weeks ($p<0.01$), egg weight ($p<0.01$) and feed intake ($p<0.05$). However, the rate of lay, egg mass and feed per egg ratio have shown non-significant differences ($p>0.05$). The association between the least squares mean of egg production traits and the *STAT5B* genotype is shown in Table 3. Body weight and reproductive traits are quantitative traits and are interrelated with complex genes and the environment[8]. From this study, it can be stated that chickens having the GG genotype are interesting for egg type chicken because they have a small body and eat so little but produce the heaviest eggs as well as AA genotype that are interesting for meat type chicken. Sadeghi *et al.*[9] and Ou *et al.*[13] have reported that *STAT5B* gene was associated with both growth and reproductive traits. This study results have demonstrated that the genetic marker of the *STAT5B* gene might be used in breed selection programs for the simultaneous improvement of traits concerning growth and egg production[9] such as in multipurpose chicken breeds.

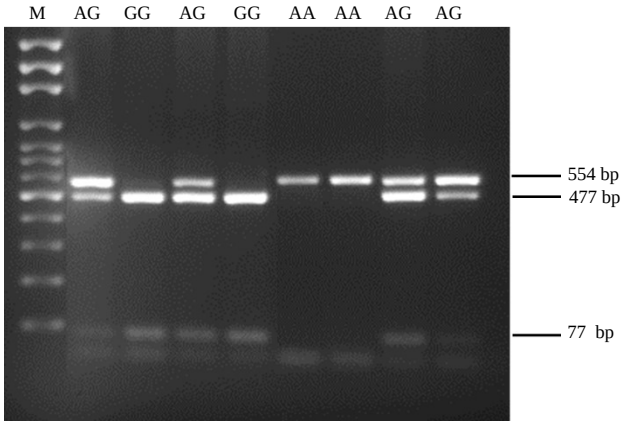

Fig. 1: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RELP) pattern of *STAT5B* gene in White Leghorn and Rhode Island Red chickens (M = 100 bp DNA ladder)

Table 2: Genotype and allele frequency of *STAT5B* gene

	Genotype frequency			Allele frequency			Heterozygosity	
Breed (N)[a]	AA	AG	GG	A	G	$(\chi^2)^b$	H_o	H_e
WLH (40)	0.351	0.108	0.541	0.405	0.595	22.267**	0.108	0.482
RIR (40)	0.525	0.400	0.075	0.725	0.275	0.001	0.400	0.399
Pooled (80)	0.438	0.254	0.308	0.565	0.435	-	-	-

[a]WLH: White Leghorn, RIR: Rhode Island Red. [b]Chi-square (χ^2) with 1 degree of freedom, the area of critical value of 6.635, the α area is 0.01 and **Significant at p< 0.01

Table 3: Association of *STAT5B* genotype on egg production traits

	STAT5B genotype			
Parameters	AA	AG	GG	SEM
Body weight at 31 weeks (kg)	1.771[a]	1.893[a]	1.496[b]	0.048**
Rate of lay (%)	72.14	69.38	64.24	1.57[ns]
Egg weight (g)	50.56[b]	46.04[b]	53.43[a]	0.87**
Egg mass (g HD^{-1})	36.34	33.37	34.40	0.91[ns]
Feed intake (g day^{-1})	84.80[a]	90.22[a]	75.55[b]	2.02*
Feed per egg ratio	2.34	2.52	2.22	0.06[ns]

[1]Different letters indicate significant difference, *Significant at p<0.05, **Significant at p<0.01 and ns: Non-significant difference at p>0.05

Table 4: Association of *STAT5B* genotype on egg quality traits

	STAT5B genotype			
Parameters	AA	AG	GG	SEM
Egg height (mm)	55.24[b]	54.18[b]	56.41[a]	0.24**
Egg width (mm)	40.86	40.68	40.83	0.30[ns]
Egg shape index (%)	74.01	74.99	72.40	1.39[ns]
Shell weight (g)	6.58[b]	6.50[b]	7.57[a]	0.15**
Shell thickness (mm)	0.33[b]	0.33[b]	0.37[a]	0.01***
Shell strength (kg cm^{-2})	3289.00	3364.00	3340.00	107.00[ns]
Albumen weight (g)	29.81[b]	29.40[b]	32.79[a]	0.43**
Albumen height (mm)	7.14	6.90	7.51	0.13[ns]
Yolk weight (g)	14.68	13.80	14.60	0.17[ns]
Yolk color	7.21[b]	7.64[a]	7.02[b]	0.06***
Haugh unit score	86.10	95.78	87.54	2.40[ns]

[1]Different letters indicate significant difference, *Significant at p<0.05, **Significant at p<0.01, ***Significant at p<0.001 and ns: Non-significant difference at p>0.05

In the association analysis between the genotype and egg quality traits, it was found that the *STAT5B* gene is associated with the traits of egg height (p<0.01), shell weight (p<0.01), shell thickness (p<0.001), albumen weight (p<0.01) and yolk color (p<0.001), but that there was non-significant difference (p>0.05) in the width of the egg shape, the strength of the egg shell, the height of the albumen, the weight of the egg yolk, or the Haugh unit (Table 4).

The physical features of the egg such as shell strength and shell thickness are some of the factors showing the quality of the egg since, when moving or transporting these eggs, their shells might be broken if they are not strong enough and resulting in economic loss[20,21]. In addition, a thicker eggshell is very useful in terms of helping to protect the egg from germs and moisture that might penetrate small holes in the eggshell. A strong eggshell positively affects the freshness and how long the egg can be kept so, improving egg shell quality is very important for the egg producer[4,20-22]. Fan *et al.*[20] reported that the sodium channel (*SCNN1*) gene family was significantly associated egg shell traits in chicken, especially egg shell strength and egg shell thickness. According to this study, it was found that the GG genotype of *STAT5B* has an egg height (p<0.01), shell weight (p<0.01) and shell thickness (p<0.001) that are higher than other genotypes. This result has shown that the *STAT5B* gene might also relate to the building process of the eggshell.

The consumer's decision to buy eggs is influenced by many factors such as price, size, freshness and the colors of the eggshell and the egg yolk (for example, a vivid egg yolk color influences the purchase decision)[22-24]. Thus, improvements in egg quality, especially the content of egg yolk have become a critical goal of layer hen breeding[25]. For this reason, many producers include more feed supplements such as xanthophyll in chicken diets. However, this study did not use feed supplements in the chicken experimental diet to augment the yolk color (Table 1) but the

result has shown that the AG genotypes of *STAT5B* have a more vivid egg yolk color than the AA and GG genotypes with a very high statistically significant difference ($p<0.001$). This study hypothesized that these variant may disturb some transcription factor-binding site thus, altering gene expression and affecting the pigment absorption or some metabolisms relating egg formation in chicken. However, this hypothesis still needs further verification.

CONCLUSION

This study is the first showing the relation between the genotype of the *STAT5B* gene and egg quality traits (egg weight, egg height, shell weight, shell thickness, albumen weight and yolk color) as well as the relation to feed intake and body weight at 31 weeks. According to the data from association analysis, it has been shown that the G allele at SNP g.4533815G>A can be one of the most important genetic markers toward improving the egg quality traits in MAS programs. The information from this study will be a useful guideline in chicken genetics with the aim of improving egg quality in order to be resistant to the tropical environment.

ACKNOWLEDGMENT

This study was supported by a grant from Naresuan University (R2558C058) and partly supported from Thailand Research Fund (TRF) through the "TRF-Research Grant for New Scholar" (MRG5580212). The researchers thank Miss Benjaphon Phusathian, Miss Chittrakhan Plerdkhunthod and all members of research group of Animal Genetic Resources, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand for their kind cooperation.

REFERENCES

1. Charoensook, R., K. Gatphayak, A.R. Sharifi, C. Chaisongkram, B. Brenig and C. Knorr, 2012. Polymorphisms in the bovine HSP90AB1 gene are associated with heat tolerance in Thai indigenous cattle. Trop. Anim. Health Prod., 44: 921-928.
2. Charoensook, R., T. Incharoen, N. Wichasit and N. Preecha, 2016. Influence of breed and sex on the growth traits of White Leghorn and Rhode Island Red chickens under topical condition. Khon Kaen Agric. J., 44: 401-404.
3. Tunim, S., M. Duangjinda and S. Katavatin, 2010. Study of *HSP 70* gene polymorphism in various strains of Thai indigenous chickens. Khon Kaen Agric. J., 38: 71-75.
4. Khawaja, T., S.H. Khan, N. Mukhtar, N. Ullah and N. Parveen, 2013. Production performance, egg quality and biochemical parameters of Fayoumi, Rhode Island Red and their reciprocal crossbred chickens. J. Applied Poult. Res., 41: 208-217.
5. Charoensook, R., 2011. Genetic Conservation and Utilization of Livestock in Northern Thailand. Cuvillier Verlag Gottingen, Germany.
6. Udy, G.B., R.P. Towers, R.G. Snell, R.J. Wilkins and S.H. Park *et al*., 1997. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc. Natl. Acad. Sci. USA., 94: 7239-7244.
7. Kofoed, E.M., V. Hwa, B. Little, K.A. Woods and C.K. Buckway *et al*., 2003. Growth hormone insensitivity associated with a *STAT5B* mutation. New Engl. J. Med., 349: 1139-1147.
8. Zhao, X.H., J.Y. Wang, G.X. Zhang, Y. Wei, Y.P. Gu and Y.B. Yu, 2012. Single nucleotide polymorphism in the *STAT5b* gene is associated with body weight and reproductive traits of the Jinghai Yellow chicken. Mol. Biol. Rep., 39: 4177-4183.
9. Sadeghi, M., S. Niknafs, H.M. Shahrbabak and S.A. Fatemi, 2012. Two SNP in *STAT5B* gene and their association with breeding value of growth and egg production traits in Mazandaran indigenous chicken. Livest. Sci., 147: 198-202.
10. Charoensook, R. and T. Pechrkong, 2016. Polymorphism of *STAT5b* gene of local indigenous chicken population in Lower-Northern Thailand. Khon Kaen Agric. J., 44: 1-6.
11. Haugh, R.R., 1937. The haugh unit for measuring egg quality. U.S. Egg Poult. Mag., 43: 552-555.
12. Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 1st Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
13. Ou, J.T., S.Q. Tang, D.X. Sun and Y. Zhang, 2009. Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. Poult. Sci., 88: 722-727.
14. Niknafs, S., A.N. Javaremi and M. Sadeghi, 2014. Single nucleotide polymorphisms in BMPR-IB and *STAT5B* genes and their association with growth and reproductive traits in chicken. Songklanakarin J. Sci. Technol., 36: 137-142.
15. Falconer, D.S. and T.F.C. Mackay, 1996. Introduction to Quantitative Genetics. 4th Edn., Benjamin Cummings, London, UK., ISBN-13: 9780582243026, Pages: 464.
16. Peakall, R. and P.E. Smouse, 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28: 2537-2539.
17. SPSS., 2010. Statistical Package for Social Sciences. SPSS Inc., Chicago, IL., USA.
18. Darnell, J.E.Jr., 1997. STATs and gene regulation. Science, 332: 1630-1633.
19. Levy, D.E. and J.E. Darnell, 2002. Stats: Transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol., 3: 651-662.

20. Fan, Y.F., Z.C. Hou, G.Q. Yi, G.Y. Xu and N. Yang, 2013. The sodium channel gene family is specifically expressed in hen uterus and associated with eggshell quality traits. BMC Genet., Vol. 14. 10.1186/1471-2156-14-90
21. Ahmadi, F. and F. Rahimi, 2011. Factors affecting quality and quantity of egg production in laying hens: A review. World Applied Sci. J., 12: 372-384.
22. Sirilaophaisan, S., P. Gunun, K. Sintala, P. Punyakaew and T. Kimprasit, 2016. Effects of dietary Mao Pomace supplementation on egg production performance, egg quality and hematology of laying hens. J. Agric., 32: 273-281.
23. Ofuosu, I.W., E. Appiah-Nkansah, L. Owusu, F.B. Apea-Bah, I. Oduro and W.O. Ellis, 2010. Formulation of annatto feed concentrate for layers and the evaluation of egg yolk color preference of consumers. J. Food Biochem., 34: 66-78.
24. Niyomdecha, A. and M. Khongsen, 2013. Metabolism and nutritional values of carotenoids on egg yolk color. Princess Naradhiwas Univ. J., 5: 112-121.
25. Sheng, Q., D. Cao. Y. Zhou, Q. Lei and H. Han *et al*., 2013. Detection of SNPs in the cathepsin D gene and their association with yolk traits in chickens. PLoS ONE, Vol. 8. 10.1371/journal.pone.0056656.