Factors affecting gene expression associated with the skin color of black-bone chicken in Thailand

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ABSTRACT The objective of this study was to investigate the effect of breed, sex, and age on the gene expression level of melanocortin 1 receptor (**MC1R**), DOPA chrome tautomerase (**DCT**), tyrosinase-related protein 1 (**TYRP1**), tyrosinase (**TYR**), and agouti signaling protein (**ASIP**) genes in Thai commercial chicken lines. All chicken have received Newscastle vaccination, and no antibiotics or any drugs were used in this study. Four chicken breeds including Black-Chinese, KU-Phuparn, Sri Mok, and Pradu Hang Dam were used in this study. These breeds can be classified by their skin color into 3 group including black (Black Chinese and KU-Phuparn), light black (Sri Mok), and yellowish white (Pradu Hang Dam). One hundred chickens per breed were used in this study. Breast skin tissue was randomly collected from 8 chickens (4 males, 4 females) per breed at 4, 8, 12, and 16 wk of age. The mRNA expression was analyzed using qRT-PCR and the gene expression level was calculated as $2^{-\Delta\Delta CT}$. From the results, breed significantly ($P < 0.01$) affected the expression level for the 5 genes evaluated. Birds with the black skin color had greater TYRP1 and TYR gene expression when compared to chickens with light black and yellowish-white skin color, respectively. Whereas, chickens with yellowish-white skin color had greater ASIP gene expression when compared to chickens having the other skin colors. Sex significantly affected DCT, TYRP1, and TYR gene expression where the gene expression in males was greater when compared to females ($P < 0.05$). Age affected all gene expression levels ($P < 0.01$). At 4 wk of age, MC1R, DCT, TYRP1, and TYR gene expression was the highest and decreased as bird age increased ($P < 0.05$); however, ASIP gene expression was greatest at 8 wk of age. After 8 wk of age all gene expression for the genes evaluated in this study decreased as age increased. In addition, an interaction between breed and sex ($P < 0.05$) impacted DCT and ASIP gene expression. The results from this study showed that all genes evaluated can be used as candidate markers to further improve the blackness of the chicken's skin because the most desired skin color is black in the Thai black-bone chicken population.

Key words: factor, gene expression, skin color, black-bone chicken

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INTRODUCTION

The black-bone chicken originated from China with a variety of breeds such as the Silky chicken, the Jinhu black-bone chicken, the Jiangshan black-bone chicken, the Lueyang black-bone chicken, the Sichuan black-bone chicken, and the Muchuan black-bone chicken (Zhu et al., 2014), which is different from broiler and layer chickens, as they have black skin and have meat and bones with unique characteristics. The poultry industry in Asia and health-minded food consumers focus on the black bone bird because of its desirable attributes including relatively high levels of bioactive compounds including melanin and carnosine, which are a powerful antioxidant (Tian et al., 2007; Tu et al., 2009). Moreover, the black-bone bird meat is reported to contain lower amounts of fat and cholesterol when compared to typical broiler chicken lines (Tian et al., 2011). In China, the black-boned chicken has been used in traditional Chinese medicine recipes for treating various diseases (Chen et al., 2009) and rehabilitating patients for thousands of years (Geng et al., 2010). Black-bone chickens can be found in many Asian countries including China, Japan, Taiwan, Vietnam, India, Indonesia, and Thailand (Chung and Chin, 2000; Hung et al., 2004; Choo et al., 2014; Rahman et al., 2014; Lukasiewicz et al., 2015). Thailand is considered a

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source for diverse black-bone chicken lines, which can be observed from the variations of phenotypes through qualitative and quantitative traits (Buranawit et al., 2016), but these chicken lines are not generally utilized in the Thai health food market. Therefore, to maximize the use of genetic resources available in Thailand for the benefit of Thai people and the consumer food sector. Using black-bone chicken as a model for functional meat production has become an interesting issue for poultry farming in Thailand. Meanwhile, it is another way to increase the value of products produced from native animal breeds under the Thai Government bioeconomy policy (Jones and Pimdee, 2017). Additionally, maintaining native animal breeds like the black-bone chicken can help support consumers in Asian countries and the world population when producing products for the health food market.

The native black-bone chicken is needed in Asian countries to produce functional meat products to meet consumer demand because of their desirable meat quality traits (Sehrawat et al., 2021). Skin color is one of the key traits the black-bone chicken possesses which is used to determine the market value of chicken (Zhang et al., 2015a) because it affects the Thai consumer's purchasing decisions. The Black-Chinese, KU-Phuparn, and Sri Mok chicken are considered black-bone chicken breeds and have been used to improve the native black-bone chicken in Thailand. Each species emphasizes different economically important traits such as growth performance, egg production, and carcass quality. Meanwhile, skin blackness is an economically important trait that consumers first consider to purchasing these birds. Additionally, darker skinned chickens have a higher value than lighter skinned chickens. Therefore, a selection approach for breeding goals is needed to control the uniformity and level of skin darkness in these chicken breeds. However, some chickens within these breeds still possess skin that appears yellowish-white and light skin color within the current breeding population, which has a direct negative economic impact on these lines. The black skin is determined by melanin levels that are synthesized by melanocytes located in the basal epidermis skin layer and melanosomes are transferred to keratinocytes (Cichorek et al., 2013). This process is controlled at some level by genetics. Several studies have reported that skin color is related to melanocortin 1 receptor (**MC1R**), DOPA chrome tautomerase (**DCT**), tyrosinase-related protein 1 (**TYRP1**), tyrosinase (**TYR**), and agouti signaling protein (**ASIP**) genes in chickens (Jiao et al., 2004; Dorshorst et al., 2010; Chi et al., 2012; Tian et al., 2014; Zhang et al., 2015b; Zheng et al., 2015; Yu et al., 2019). These genes regulate the melanogenesis pathway. However, previous research has reported that some genes were not different when examining differential expression between chickens that have the yellowish-white and black skin color (Zhang et al., 2015a). Recent studies have reported that some genes controlling skin color may be population-specific (Zhang et al., 2015a; Yu et al., 2019). As a result, these genes may not be informative when used in other populations. Therefore, understanding the basic profiles for genes determining skin color is important to developing genetic markers to be used in selection programs designed genetically improve bird skin color.

The aim of this study is to investigate the effects of breed, sex, and age on the profile of genes and associated gene expression related to skin color in Black-Chinese, KU-Phuparn, and Sri Mok chicken breeds. The genetic marker information can be used to improve the blackness of skin in the black-bone chicken population in Thailand.

MATERIALS AND METHODS

Ethics Statement

This study was conducted in strict according to the Guideline for the Care and Use of Laboratory Animals of the National Research Council of Thailand. The protocol of animal experimentation was approved by the Institutional Animal Ethics Committee, Khon Kaen University (Approval Number: IACUC-KKU-35/62).

Animals Management

Four breeds of chicken were evaluated in this study and included Pardu Hang Dam (**PD**, Thai native chicken),

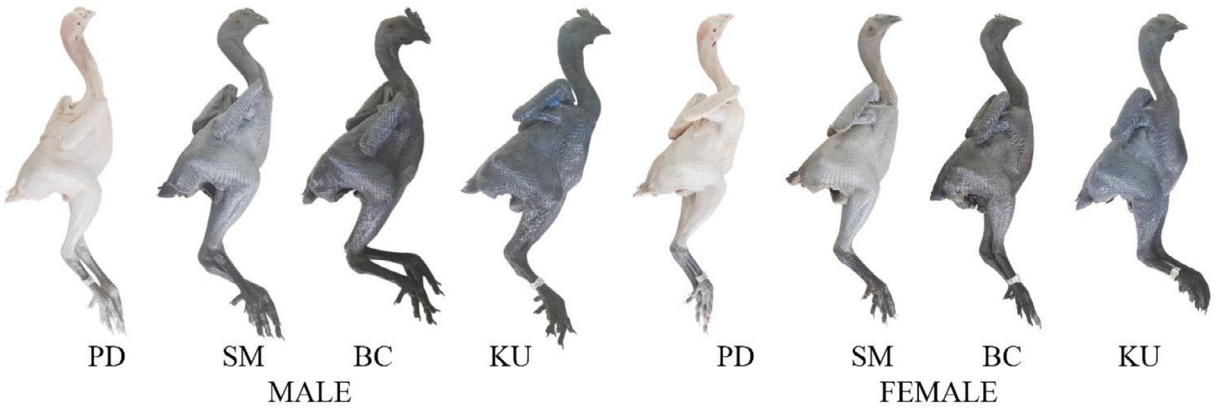

Figure 1. Chicken skin color[2] at 12 wk of age[3] classified into 3 skin color categories by breed[1] and sex. [1]PD (Pradu Hangdam) is the Thai native chicken; SM (Sri Mok) is crossbred between Thai native chicken and black-bone chicken; BC (Black-Chinese) and KP (KU-Puparn) are the black-bone chicken. [2]yellowish-white (PD), light black (SM), and black (BC and KP) skin colors. [3]These breeds reach the market weight at 12 wk of age.

Black-Chinese (**BC**, Black bone chicken), Sri Mok (**SM**, Pardu Hang Dam × Black-Chinese), and KU-Phuparn (**KP**, Black bone chicken) chicken lines (Figure 1). Pardu Hang Dam, Black-Chinese, and Sri Mok were obtained from the Research and Development Network Center for Animal Breeding (Native Chickens), Faculty of Agriculture, Khon Kaen University and KU-Phuparn was obtained from the Animal research farm of Kasetsart University Chareamprakiat Sakon Nakorn Campus. In total, 100 one-day-old chicks per breed were used in this study. The chicks from each breed were housed in 4 pens of 25 chickens each. Chickens were raised at Research and Development Network Center for Animal Breeding (Native chicken) farm, Faculty of Agriculture, Khon Kaen University and provided ad libitum access to diets formulated to meet the nutritional requirements for the native chicken: first period 21% CP, 3,200 kcal ME/kg (0 −4 wk) and second period 18% CP, 3,200 kcal ME/kg (4 −16 wk). The chickens were reared under typical conditions used by farmers that utilize the open production system in the tropical climate experienced in Thailand. All chicken have received Newscastle vaccination, and no antibiotics or any drugs were used in this study. The size of the experimental pens where the chicks were raised was 2 × 2 m. The density was approximately 6.25 chicks per square meter for all 16 pens used in this study. Two chickens (1 male and 1 female) per pen or 8 chickens per breed were randomly selected to be harvested at 4, 8, 12, and 16 wk of age from each breed. In total, 32 chickens per wk were harvested following feed withdrawal for 12 h before harvest. The chickens were euthanized and dressed following the Thai processing style (Jaturasitha, 2004). The breast skin tissue samples were isolated and snap-frozen in liquid nitrogen and stored at −80°C until further laboratory processing and analysis occurred.

RNA Extraction

Total RNA was extracted from frozen breast skin tissue (200 mg) samples using GeneJET RNA Purification Kit (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. The RNA purity and concentration were verified using a spectrophotometer (NanoDrop 2000 /2000c, Thermo Fisher Scientific Inc.) where absorbance was measured at 260 and 280 nm. The extracted RNA concentration had at least 100 ng/μL and typically good-quality RNA should have an A260/A280 ratio greater than 1.9, indicating that the RNA is high purity (Dash, 2013).

Quantification of mRNA Expression

The one-step quantitative reverse transcription PCR (**qRT-PCR**) technique was used to measure gene expression level using Bio-Rad CFX96 Touch Real-Time PCR Systems (Bio-Rad, Hercules, CA). The reaction was performed in a volume of 20 μL containing 10 μL of 2x SensiFAST SYBR No-ROX One-Step Mix (Bioline, Memphis, TN), 1 μL (400 nM) of each primers, 0.2 μL Reverse transcriptase, 0.4 μL RiboSafe RNase Inhibitor, 3.4 μL DEPC-treated water and 4 μL (20 ng/μL) of RNA template. The cycling condition consisted of cDNA synthesis step at 50°C for 10 min, a polymerase activation at 95°C for 5 min, then PCR cycling 40 cycles of 95°C for 10 s, the 58°C for 25 or 30 s and melting curves were generated at 65°C to 95°C. Each sample was performed in duplicate. 18S rRNA was used as a housekeeping gene. Gene expression levels were calculated via the $2^{-\Delta\Delta CT}$ methods (Livak and Schmittgen, 2001). Gene-specific primers were designed by using Primer-BLAST NCBI and the primer sequences are described in Table 1.

Statistical Analysis

Gene expression data were analyzed using linear model methods to investigate the effects of breed (4 breeds), sex (male and female), age (4, 8, 12, and 16 wk), and their interactions. When significant effects were detected, mean comparisons were conducted using Tukey's multiple range test ($P < 0.05$). The impact that age had on gene expression was evaluated using preplanned orthogonal polynomial comparisons (SAS. 2019).

Table 1. Gene and primer sequences used for qRT-PCR in a study of skin color[2] among four chicken breeds[1] in Thailand.

Gene	Primer sequence (5'–> 3')	Chr[5]	GeneBank ID[6]	Product size (bp)[7]
MC1R[3]	F: ATCCTGCTCTGCCTCATTGG R: TAGATGGTGGGCTGCTTCTG	11	NM_001031462.1	131
TYR[3]	F: AGGCGACTGAGAACGAGAAG R: TGCATCCAGACGAAGAGATCG	1	NM_204160.1	197
TYRP1[3]	F: TGGCCTACCCTACTGGAACT R: CACACGCCACGTAGAGAAGA	Z	NM_205045.1	133
DCT[3]	F: TTCAGCTTCAGGAATGCGCT R: CTGCGTGAGGGAGAACTCTG	1	NM_204935.1	124
ASIP[3]	F: CTCCAAAACCAAGGCCCACA R: GGCATTTGCACAACGCACAG	20	NM_001115079.1	105
18S rRNA[4]	F: CGGCGACGACCCATTCGAAC R: GAATCGAACCCTGATTCCCCGTC	16	XR_003078044.1	99

[1]Breeds of chicken: Black-Chinese, KU-Phuparn, Sri Mok, and Pradu Hang Dam.
[2]Skin color groups: black (Black Chinese and KU-Phuparn), light black (Sri Mok), and yellowish-white (Pradu Hang Dam).
[3]Melanin related gene.
[4]Housekeeping gene in this study was universal primer (Fenwick et al.,2008).
[5]Chr = Chromosome.
[6]GeneBank ID is an accession number of a DNA sequence in GeneBank database.
[7]Product size (bp) = PCR product size (base pair).

RESULTS AND DISCUSSIONS

Effect of Breed on Gene Expression Level

The Black-Chinese and KU-Puparn chicken breeds had greater MC1R, DCT, TYRP1, and TYR gene expression when compared to Sri Mok and Pradu Hangdam (3−8 fold) ($P < 0.01$). The Pradu Hangdam chicken breed had the greatest ASIP gene expression (2−5 fold) when compared to other breeds ($P < 0.01$) (Table 2). From the present experiment, it was found that Pradu Hang Dam with yellowish-white skin had the lowest MC1R, DCT, TYRP1, and TYR gene expression in this gene group ($P < 0.01$). The Sri Mok chicken breed with light black skin, which is a crossbred between yellowish-white and black skin chicken lines, was found to have moderate MC1R, DCT, TYRP1, and TYR gene expression, which was lower than the chicken breeds with black skin, but greater when compared to the chicken line with yellowish-white skin. These results are consistent with published research that evaluated the gene expression for the same genes used in this study and that involved black-bone chickens from different populations (Luo et al., 2013; Zhang et al., 2015a,b; Zheng et al., 2015). The DCT, TYRP1, and TYR genes have been reported to regulate enzyme production (Dopachrome tautomerase, Tyrosinase-related protein 1, Tyrosinase) related to melanogenesis (Yasumoto et al., 2002; Jiao et al., 2004) which is consistent with the results from the present study. Results from the current study indicated that the Black-Chinese and KU-Puparn chicken breeds with black skin had greater DCT, TYRP1, and TYR gene expression when compared to other breeds with yellowish-white and light black skin. The MC1R (Melanocortin 1 receptor) gene has been reported to play a role in regulating skin and hair color by controlling enzyme activity involved in melanogenesis (Zhang et al., 2015a,b). Other studies have reported that chickens with black skin had a greater level of MC1R gene expression when compared to the chicken breed having yellowish-white skin (Liu et al., 2010; Zhang et al., 2015b) which is consistent with the results from the present study. In the present study, both black-bone chicken breeds showed greater gene express levels for MC1R gene when compared to the Pradu Hang Dam chicken breed. In addition, no MC1R, DCT, TYRP1, TYR gene expression differences were observed between Sri Mok and Pradu Hang Dam chicken breed ($P > 0.01$), but there were differences in skin appearances between the breeds. The fact that there appeared to be skin color differences between the Sri Mok and Pradu Hand Dam breeds may be due to the interaction between proteins and enzymes involved in the melanin synthesis process, resulting a reduction or inhibition of enzyme activity (Sitaram and Marks, 2012; Wang et al., 2012). Therefore, the gene mutation that regulates these binding proteins can affect skin color, which should be evaluated in future studies. The ASIP (agouti signaling protein) gene has been reported to play an important role in determining the skin color in animals. This gene is responsible for preventing α-MSH binding to MC1R, resulting in inhibition eumelanin synthesis (Lu et al., 1994). Zhang et al. (2015) reported that the yellowish-white skin chicken had a greater level ASIP gene expression when compared to black skin chickens. Additionally, previous studies have shown that SNP points were located in the promoter region which determines the gene expression variation that results in the different skin color in the chicken (Yu et al., 2019). The results of this study are consistent with the previous studies, where the expression of this gene in Pradu Hang Dam chicken breed was greater when compared to the other breeds used in this study.

Table 2. Effect of breed and age on the gene expression level ($2^{-\Delta\Delta CT}$) of MC1R, DCT, TYRP1, TYR, and ASIP genes in four breeds[1] involved in a study of chicken skin color[2] in Thailand.

	Gene[3]				
Effect	MC1R	DCT	TYRP1	TYR	ASIP
Breed					
- Pradu Hangdam	0.72^{b}	0.99^{c}	0.69^{b}	0.65^{b}	1.08^{a}
- Sri Mok	1.32^{ab}	1.81^{bc}	1.41^{b}	0.85^{b}	0.64^{b}
- Black-Chinese	2.00^{a}	3.41^{a}	5.89^{a}	3.16^{a}	0.26^{b}
- KU-Puparn	1.88^{a}	2.58^{ab}	3.64^{a}	3.69^{a}	0.26^{b}
SEM	0.26	0.40	0.83	0.65	0.14
P-value[4]	0.0025	0.0004	0.0022	0.0012	0.0001
Age					
- 4 wk	2.66^{a}	2.72^{a}	4.22^{a}	3.13^{a}	0.74^{a}
- 8 wk	1.77^{b}	2.58^{a}	2.71^{b}	2.53^{ab}	0.91^{a}
- 12 wk	1.01^{bc}	2.15^{ab}	1.98^{bc}	1.76^{ab}	0.52^{a}
- 16 wk	0.49^{c}	1.33^{b}	0.86^{c}	0.93^{b}	0.06^{b}
SEM	0.26	0.40	0.53	0.65	0.14
P-value[4]	<0.0001	0.0021	0.0032	0.0034	0.0003

^{a,b,c}Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Breeds of chicken: Black-Chinese, KU-Phuparn, Sri Mok, and Pradu Hang Dam.

[2]Skin color groups: black (Black Chinese and KU-Phuparn), light black (Sri Mok), and yellowish-white (Pradu Hang Dam).

[3]MC1R, melanocortin 1 receptor; DCT, DOPA chrome tautomerase; TYRP1, tyrosinase-related protein 1; TYR, tyrosinase; and ASIP, agouti signaling protein.

[4]The *P*-values were determined from GLM.

Effect of Sex on Gene Expression Level

Gender was found to affect DCT, TYRP1, and TYR gene expression levels ($P < 0.05$; Table 3) which regulate enzyme production involved in melanin synthesis. The present study observed that male chickens had a greater gene expression level for these genes when compared to female chickens ($P < 0.05$). In the past, the genetic improvement of the black-bone chicken population in Thailand included skin color as one of the breeding goals. Normally, the selection intensity in the male is greater than females. The chicken with the highest level of black skin color is typically selected as a breeder for the next generation. Therefore, the selection intensity may result in the greater expression of genes controlling black skin color in male chickens when compared to female chickens in this study. This result contrasted with Zhang et al. (2015b) reported that male black-boned chickens had a lower TYRP1 gene expression when compared to female. In addition, Tadokoro et al. (2003) illustrated that the

Table 3. Effect of sex and sex across all of breed on the gene expression level ($2^{-\Delta\Delta CT}$) of MC1R, DCT, TYRP1, TYR, and ASIP genes in four breeds[1] involved in a study of chicken skin color[2] in Thailand.

		Gene[3]				
Breed	Sex	MC1R	DCT	TYRP1	TYR	ASIP
Pooled breeds	Male	1.66	2.71^{a}	4.39^{a}	2.88^{a}	0.58
	Female	1.30	1.69^{b}	1.42^{b}	1.29^{b}	0.54
	SEM	0.18	0.29	0.73	0.46	0.09
	P-value[4]	0.1991	0.0165	0.0052	0.0191	0.6809
Pradu Hangdam	Male	0.76^{c}	0.78^{c}	0.88^{c}	0.59^{c}	0.70^{bc}
	Female	0.68^{c}	1.17^{c}	0.51^{c}	0.70^{c}	1.42^{a}
Sri Mok	Male	1.37^{abc}	2.17^{bc}	1.56^{bc}	0.95^{c}	0.78^{b}
	Female	1.27^{bc}	1.40^{c}	1.25^{c}	0.75^{c}	0.47^{bc}
Black-Chinese	Male	2.38^{a}	4.80^{a}	9.53^{a}	5.07^{a}	0.34^{bc}
	Female	1.62^{abc}	2.03^{bc}	2.24^{bc}	1.24^{c}	0.17^{c}
KU-Puparn	Male	2.08^{ab}	2.99^{b}	5.56^{ab}	4.89^{ab}	0.31^{bc}
	Female	1.68^{abc}	2.17^{bc}	1.73^{bc}	2.49^{bc}	0.20^{c}
	SEM	0.36	0.56	1.46	0.88	0.20
	P-value[4]	0.0136	0.0001	0.0002	0.0001	0.0002

[a,b,c]Means within a row lacking a common superscript differ ($P < 0.05$).
[1]Breeds of chicken: Black-Chinese, KU-Phuparn, Sri Mok, and Pradu Hang Dam.
[2]Skin color groups: black (Black Chinese and KU-Phuparn), light black (Sri Mok), and yellowish-white (Pradu Hang Dam).
[3]MC1R, melanocortin 1 receptor; DCT, DOPA chrome tautomerase; TYRP1, tyrosinase-related protein 1; TYR, tyrosinase; and ASIP, agouti signaling protein.
[4]The *P*-values were determined from GLM.

testosterone hormone influenced the cAMP reduction levels and tyrosinase activity within melanocyte, but did not affect the gene expression level resulting in reduced melanin synthesis which was studied in the human melanocyte. Currently, the effect of sex on skin color in chickens needs more investigation to provide greater clarity in its role in determining skin color.

Effect of Age on Gene Expression

The change of age in the range of 4 to 16 wk used in the present study was found to significantly affect gene expression in skin tissue ($P < 0.01$; Table. 2). At 4 wk of age, MC1R, DCT, TYRP1, and TYR gene expression was the greatest when compared to other ages evaluated in this study. However, the ASIP gene expression was the greatest when the chicks reached 8 wk and then declined at the older ages evaluated in this study. The gene expression response as age increased in the present study for MC1R, DCT, TYRP1, and TYR genes decreased linearly ($P < 0.01$) and the ASIP gene expression level followed a quadratic pattern ($P < 0.05$; Figure 2). The findings for the genes evaluated in this study are consistent with Zhang et al. (2015b) who studied the skin tissue from the Silkie chicken breed and reported that at 4 wk of age, MC1R and TYRP1 gene expression levels were greatest and decreased at 8, 12, and 16 wk of age, respectively. In addition, Liu et al. (2010) evaluated the Dongxiang black chicken breed skin color from hatching to 112 d and reported that TYR gene expression was greatest at the hatching, and the MC1R gene expression was the greatest at 28 d of age and subsequently decreased. From reducing the expression for genes involved with melanin synthesis, results in changes in melanin accumulation in skin tissue. Zhang et al. (2015b) evaluated skin tissue from the Dongxiang black chicken breed and a commercial layer breed and reported that the skin color constantly faded as the chickens age increased. The cause for the change in skin color may be due to the difference in the number of melanocytes at different ages. Nishimura et al. (2006) studied the Silkie chicken breed and reported that age affected the number of melanocytes in muscle tissue which found that as chicken becomes older, the number of melanocytes decreased, which may be related to the α-MSH hormone in the keratinocyte. From the present study, the skin tissue from chickens did not provide enough information to explain this phenomenon clearly. Previously, many studies involving human tissue reported that gene expression related to skin and hair color decreased with increasing subject age and was associated with genes related to the development, the melanosome transport, the aging process, and the apoptosis of the melanocyte cells, causing skin and hair color changes (Whiteman et al., 1999; Cichorek et al., 2013). Therefore, the different gene expression level at each age may be due to factors involving other genes or hormones, resulting in the genes evaluated in the present study to be differentially expressed at different ages. Additionally, all of the chickens in the present study were harvested at relatively young ages when compared to studies involving humans where old age is defined in years.

Interaction Breed and Sex on Gene Expression Level

From the evaluation of breed and sex in the present analysis, it was found that significant differences ($P < 0.05$) for DCT and ASIP gene expression levels (Table 4). This result from the present study found that sex of the chicken resulted in different gene expression levels within each breed (Figure 3).

The result from the present study showed that in male Black-Chinese breed had the greatest gene expression when compared to other factor combinations (breed × sex) ($P < 0.05$) at 16 wk of age, and male and female Pradu Hangdam had the lowest gene expression at 8, 12, and 16 wk of age in DCT gene that related melanin synthesis enzyme. In addition, ASIP gene expression, which is involved in the inhibition melanin synthesis pathway in female Pradu Hangdam, was the greatest when compared to other factor combinations ($P < 0.05$) at 4 and 8 wk of age. Additionally, it was observed that the black skin chicken (Black-Chinese and KU-Phuparn) had greater DCT gene expression when compared to chickens with light black skin chicken (Sri Mok) and yellowish-white skin color (Pradu Hangdam). Whereas, the chicken with yellowish-white skin had greater ASIP gene expression when compared to the black skin and the light black skin chicken. The ASIP gene expression differences may result from the fact that each chicken breed may have a different genetic basis

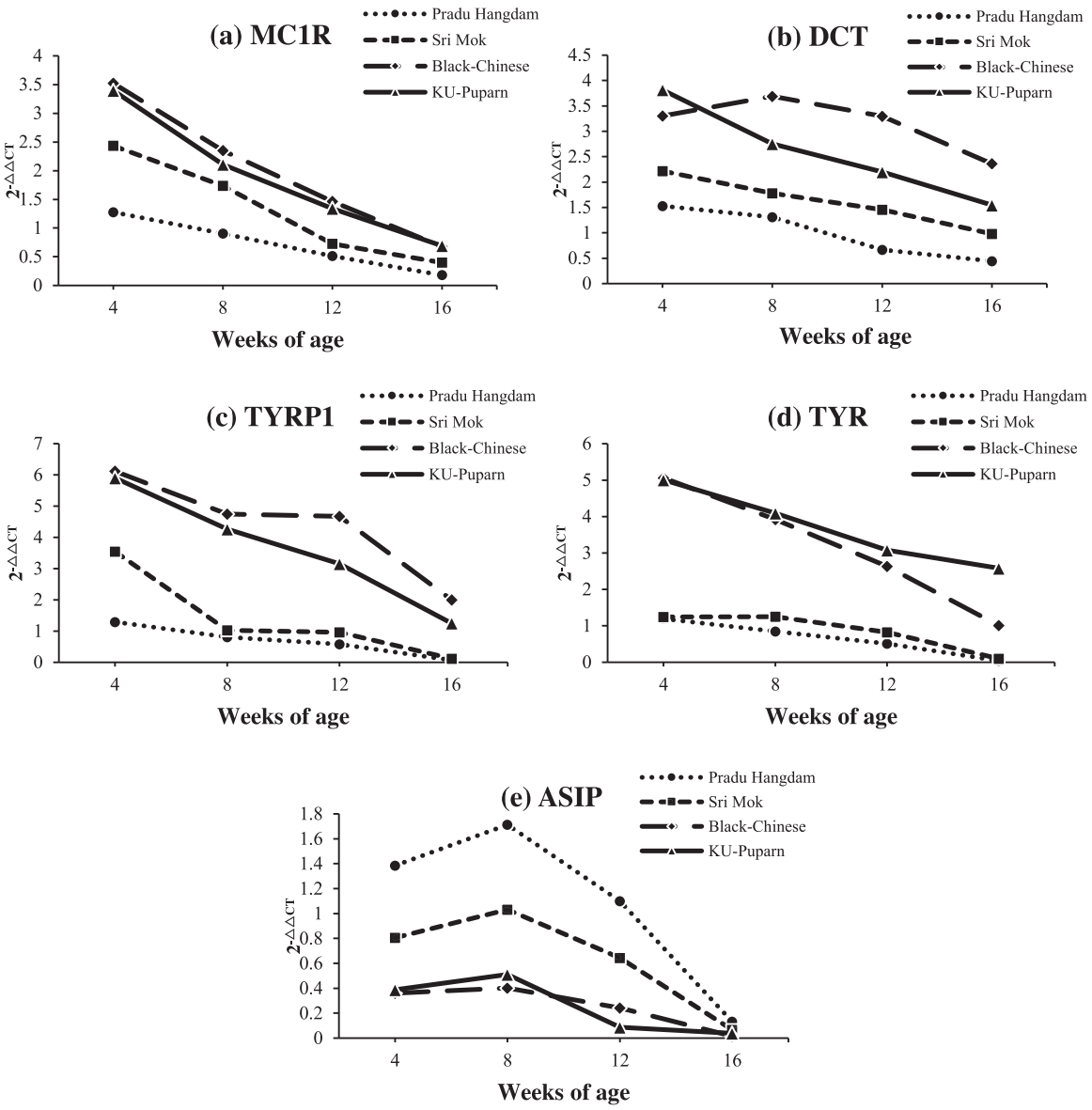

Figure 2. Gene[3] expression response trend levels with increasing ages from 4 to 16 wk of age in four chicken breeds[1] used in a study to evaluated skin color[2]. [1]Breeds of chicken: Pradu Hang Dam, Sri Mok chicken, Black-Chinese, and KU-Phuparn. [2]Skin color groups: black (Black Chinese and KU-Phuparn), light black (Sri Mok) and yellowish-white (Pradu Hang Dam). [3]MC1R, melanocortin 1 receptor; DCT, DOPA chrome tautomerase; TYRP1, tyrosinase-related protein 1; TYR, tyrosinase; and ASIP, agouti signaling protein.

and differing breeding goals to improve economically important traits such as growth performance, egg production, and carcass quality. The blackness skin in Sri Mok chicken, Black-Chinese chicken, and KU-Phuparn chicken is one of the main breeding objectives.

Table 4. The *P*-value[1] of interaction for the breed, sex, and age influences the gene expression level of MC1R, DCT, TYRP1, TYR, and ASIP genes.

	Gene[2]				
Effect	MC1R	DCT	TYRP1	TYR	ASIP
Breed × Sex	0.7686	0.0494	0.5479	0.1060	0.0183
Breed × Age	0.9031	0.9170	0.6023	0.9915	0.6676
Sex × Age	0.8831	0.1651	0.2431	0.5841	0.3356
Breed × Sex × Age	0.1073	0.4012	0.4107	0.5783	0.1658

[1]The *P*-values were determined from GLM.
[2]MC1R, melanocortin 1 receptor; DCT, DOPA chrome tautomerase; TYRP1, tyrosinase-related protein 1; TYR, tyrosinase; and ASIP, agouti signaling protein.

CONCLUSIONS

In summary, breed, sex, and age affect expression for genes contributing to skin color in the Black-bone chicken population used in this study. The results show that bird breeds with the black skin color had greater expression of TYRP1, TYR genes when compared to breeds with light black and yellowish-white skin color. Meanwhile, birds with yellowish-white skin color had greater ASIP gene expression when compared to chickens having light black and yellowish-white skin color. The expression of DCT, TYRP1, and TYR gene expression in males was greater when compared to females. It

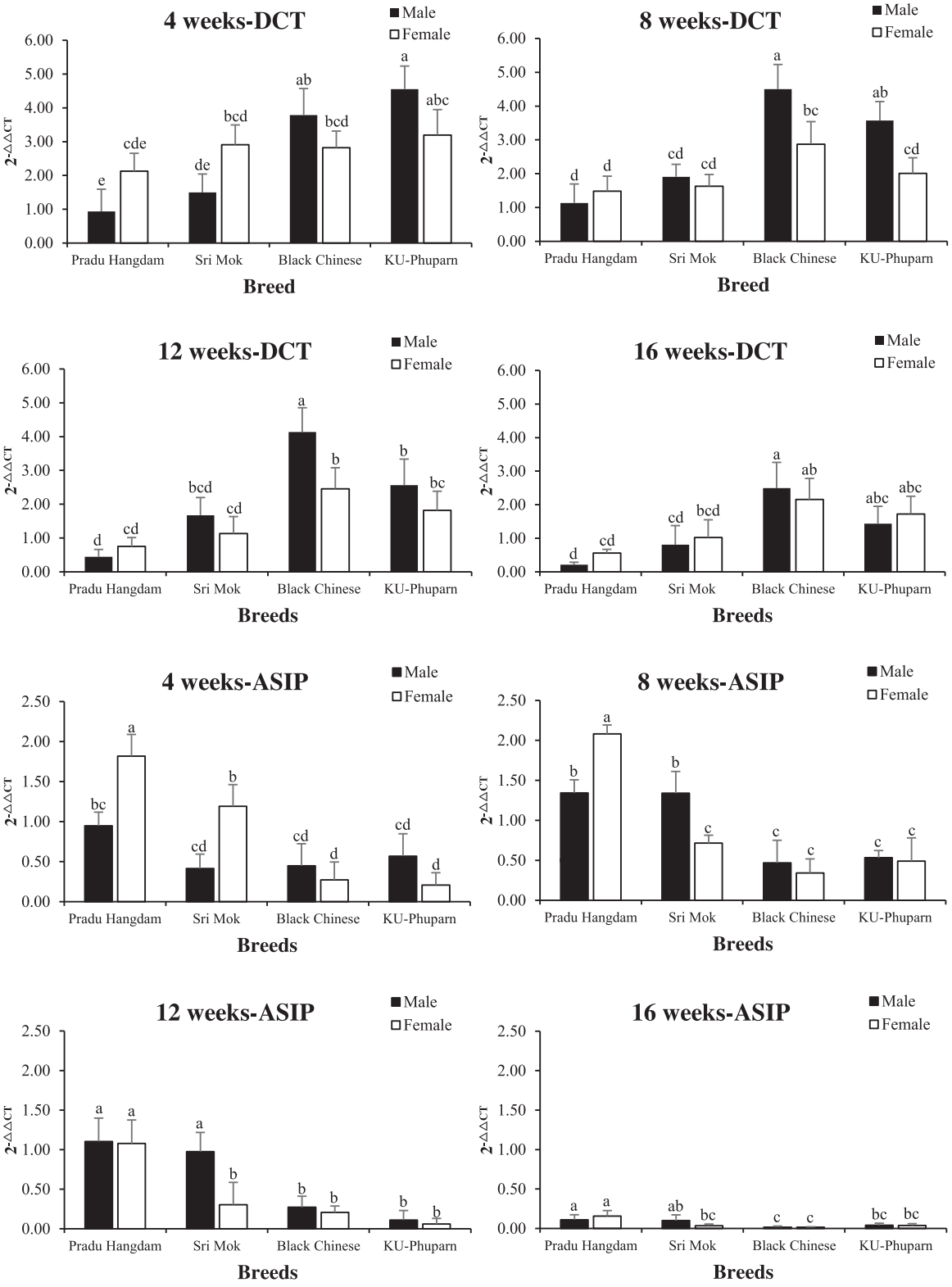

Figure 3. Breed by sex interaction effects for DCT[3] and ASIP[3] gene expression levels from four chicken breeds[1] used in a study evaluating skin color[2]. [a,b,c] Bars lacking a common superscript differ ($P < 0.05$), using Proc GLM, SAS. [1]Breeds of chicken: Black-Chinese, KU-Phuparn, Sri Mok chicken, and Pradu Hang Dam. [2]Skin color groups: black (Black Chinese and KU-Phuparn), light black (Sri Mok), and yellowish-white (Pradu Hang Dam). [3]DCT, DOPA chrome tautomerase; and ASIP, agouti signaling protein.

also showed that gene expression levels decreased with increasing age in all the genes in this study. In addition, it is known that MC1R, DCT, TYRP1, TYR, and ASIP genes contribute to skin color variation in the Thai chicken populations. Recently, many reports on different chicken populations that were included this study showed that some genes do not significantly impact skin color. Therefore, the results from the present study confirm these genes could be used as candidate genes to create DNA markers for use as a selection tool to improve

the unique black skin color in the Black-bone chicken population. Improving the black skin color will help poultry producers meet consumer demands for meat and health products produced from this chicken population.

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DISCLOSURES

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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