Expression of Glucocorticoid receptor (NR3C1) gene in 3 different breeds of Thai chickens

Menghak Phem¹, Monchai Duangjinda¹,²*, Yupin Phasuk¹,², Natthaya Duanghaklang¹ and Kanob Sujikara³

ABSTRACT: The objective of the study was to identify the expression level of a gene related to stress codifying for glucocorticoid receptor (NR3C1) in three different breeds; Chee KKU12, Khai Mook Esarn KKU50, and native-crossing commercial (Tanaosree) chickens under the same regime of management. The blood samples were collected at week 4, 8 and 12 from the three breeds by replicating 10 chickens per breed for RNA extraction. To measure the expression level of targeted gene, one-step quantitative real-time PCR (qRT-PCR) was used. The results demonstrated that the level expression of NR3C1 in 3 breeds are significantly different (P<0.01) and this was also significantly different in age of blood sampling at week 4, 8 and 12 (P<0.01). At the 12th week, it showed the highest expression level of NR3C1 gene and was quite different amongst the breed types when Tanaosree chicken showed the highest expression from week 8 and peaked at week 12. However, there was no interaction between the age of blood sampling and breed types (P>0.05). The current study demonstrates that the breeding improvement by producing synthetic breed and the native-crossing commercial breed has an effect on the selection response to NR3C1 gene and these 2 breed lines has a lower resistant ability to handle stress than Chee KKU12 which is 100% Thai native Chicken.

Keywords: Gene expression, synthetic breed, native-crossing commercial chicken, Thai native chicken

Introduction

Chicken produces white-meat that is preferentially consumed and economically thriving for the country. According to the current trend of chicken production, the indigenous chicken breed is under development to be a production system which is more welfare-friendly and harmless to the environment (Duangjinda, 2015).

However, there are some stressors that may occur in the poultry production either in tropical or sub-tropical countries. The primary one is found to be involved with high-temperature stress or heat stress (Cahancer et al., 2008). Rimoldi et al. (2015) and Lin et al. (2006) demonstrated that when the animal receives the state of stress associated high environmental temperature; it can affect the health of animal and lead to suppressing the growth, productivity, resulting in high mortality rate which has an adverse effect on the poultry producers’ profitability.

The stressful condition can activate the hypothalamus-pituitary-adrenal axis resulting in the consequent secretion of steroid hormone namely glucocorticoid (GC) from adrenal glands (Zona fasciculata and Zona reticularis cells) (Rose et al., 2010). The glucocorticoid contains mainly cortisol has the effects in body development, metabolism, immunological responses

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(immunosuppressive) and inflammation responses (Anti-inflammatory) (Ramamoorthy and Cidlowski, 2013). The glucocorticoid has receptor known as nuclear receptor subfamily 3, group c, member 1 (NR3C1) and the activation of NR3C1 up-regulates gene expression for anti-inflammatory in the nucleus and repression for pro-inflammatory in the cytosol (Kwok et al., 2007).

The study on mammals found that there was the genetic difference in relation to the production of adrenocorticotropic hormone and different adrenal cortex origin (Desautes et al., 1999; Gomez et al., 1996). The different genetic backgrounds in the hypothalamus-pituitary-adrenal (HPA) axis response to stress possibly due to the differences in the development of physiological and the differences in the coping ability (Veenema et al., 2003). Furthermore, this was clarified by Marelli et al. (2010) who claim that the GR gene expression demonstrated significant differences in three different Italian chickens. In addition, the selection for growth performance in chickens may reduce based on its response to stress (Soleimani et al., 2008). Hence, the expression of NR3C1 gene in native, synthetic, and native-crossing commercial chickens may be different. Though NR3C1 are influenced by any stressors, nothing is known about breed variation in response to the same management.

Therefore, the objective of this study aims to investigate the expression of genes codifying for glucocorticoid receptor (NR3C1) in 3 different breeds; Chee KKU12, Khai Mook Esarn KKU50, and native-crossing commercial chickens (Tanaosree) chickens, and Thai native chickens are expected to have a coping ability to handle stress in the same management.

### Materials and Methods

#### Animals and animal care

The experiment was done on by 3 different breeds; 100% Thai native chickens (Chee KKU12), Thai synthetic chickens (Khai Mook Esarn KKU50), and native-crossing commercial chickens (Tanaosree Thai chicken). Khai Mook Esarn KKU50 breed is a Thai synthetic breed bred by cross-breeding between Chee KKU12 (sire line) and commercial chickens of Tanaosree strain named “Likit chicken” (dam line). Both 100% Thai native and Thai synthetic chickens were received from breeding stocks of Research and Development Network Center for Animal Breeding (Native Chicken), NCAB, Khon Kaen University, Thailand.

Tanaosree Thai chickens (Official name “Tanaosree”) were distributed by a commercial company in Thailand, Tanaosree Thai chicken Co. Ltd. This breed was developed by mixing the genes from traditional Tanaosree chickens (sir line) and “Likit chicken” (Dam line), selecting genes that will result in rapid growth, reduced feeding time, high meat quality and beautiful body. Both Khai Mook Esarn KKU50 and Tanaosree are 50% native chicken (Table 1).

<table>
<thead>
<tr>
<th>N°</th>
<th>Breed</th>
<th>Sire line</th>
<th>Dam line</th>
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<tbody>
<tr>
<td>1</td>
<td>Chee KKU12 (Native 100%)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>Khai Mook Esarn KKU50</td>
<td>Chee</td>
<td>Likit</td>
</tr>
<tr>
<td>3</td>
<td>Tanaosree</td>
<td>Tanaosree native chicken</td>
<td>Likit</td>
</tr>
</tbody>
</table>
All birds were given access to water and fed ad libitum following the regime of standard broiler diet and existing guidance of NCAB. They were placed in the opening wire floor house containing 60 heads/breed separated to 3 replications; all replications of each breed were placed randomly as Completely Randomized Design (CRD). The chicks were brooded for 21 days and kept for 7 days to adapt before the experiment began. All birds were vaccinated with an inactive infectious Newcastle disease (ND) and Infectious Bronchitis (IB) at 1-day old and repeated again at the 4th week of age, and they were given orally with an anti-parasite drug at week 6.

**Sample and extraction of total RNA**

Blood samples from Chee KKU12 (N=10), Khai Mook Easrn KKU50 (N=10) and Tanaosree (N=10) were collected. Blood collection was repeated on the 4-week, 8-week and 12-week old chicks for total RNA extraction. Blood was collected on EDTA-NA$_2$-treated collection tube at 4°C. Total RNA was extracted from white blood cell by GeneJET RNA Purification Kit (Thermo Scientific). The quantity was measured using spectrophotometer (NanoDrop 2000 Thermo Scientific, Waltham, MA, USA) and stored at -20 °C for use in quantitative real-time PCR.

**Quantitative real-time PCR**

To study gene expression, the molecular technique namely quantitative real-time polymerase chain reaction (qRT-PCR) was applied. The primer sequence of the targeted gene--NR3C1 was used following Rimoldi et al. (2015). 18-Subunit Ribosomal RNA gene (18S-RNA) was considered as the endogenous control gene in the qRT-PCR and the primer sequence taken from Fenwick et al. (2008) (Table 2).

### Table 2 The Primers (Forward and Reverse primers) for q-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide sequence (5′-3′)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>NR3C1</td>
<td>F: GCAGCTGCAAGTGTCTTCCAAAA</td>
<td>Rimoldi et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>R: GTTCTCTCCAGGAGAGATAG</td>
<td></td>
</tr>
<tr>
<td>18SrRNA</td>
<td>F: CGGCGAGCAGCCATTCAAC</td>
<td>Fenwick et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>R: GAATCGAACCCTGATTTCCGTC</td>
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qRT-PCR was conducted using CFX96 real-time system (BIOLINE). Total volume of reaction was performed for 20 μl which containing 2 ng/μl of total RNA, 18 μl of master mix including 10 μl 2X SYBR green RT-PCR Reaction Mix (BIOLINE), 2 μl (3 μM) of primers (Forward and Reverse), RNase inhibitor 0.4 μl, 0.2 μl of iScript Reverse transcriptase for one-Step RT-PCR (BIOLINE) and 3.4 μl nuclease free water (DEPC). Incubate complete reaction mix in a real-time thermal detection system as follows: cDNA synthesis 10 min at 50°C, iScript Reverse transcriptase inactivation 5 min at 95°C, PCR cycling and detection (40 cycles): 10 sec at 95°C combining with an annealing step at 15 sec at 60°C. To minimize error from a pipette, master mixes were prepared to set up duplicate reactions for each sample.
**Statistical analysis**

The relative quantification of gene expression was computed by using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). Relative normalized expression ($2^{-\Delta C_T}$) data were analyzed by the repeated measurement split-plot in time using PROC ANOVA procedures of SAS (SAS Institute, 1996) at a significance based on the 0.05 level of probability. Breed groups (Chee KKU12, Khai Mook Esarn KKU50, and Tanaosree) were used as the main plot and the week of blood collection (4th, 8th, 12th week) used as sub-plot in the model.

**Results and Discussion**

Based on the qRT-PCR study, the result demonstrates that expression of the NR3C1 gene was significantly different between breed ($P<0.01$). When Tanaosree had the highest expression, and 100% Thai native chicken, Chee showed the lowest expression as shown in Figure 1.

![Figure 1](image)

**Figure 1** Log$_2$ fold change in Glucocorticoid receptor (NR3C1) gene expression relative to the control gene (18S-RNA) due to individual ability among three different breeds

Chee KKU12 is 100% Thai native chicken, which can adapt well in domesticated area, has slow-growing strains. Khai Mook Esarn KKU50 and Tanaosree are 50% native, which was developed to be more suitable for commercial intensive production. The study found that the synthetic line tended to express NR3C1 gene higher than slow-growing breed and native-crossing commercial line, expressed the highest level among other breeds ($P<0.001$) (Figure 1).

The environmental factor such as ambient temperature in raising system, stocking density, and nutritional factor can cause the chicken to activate the stress. The stressful situation was found to be significantly associated with activation of hypothalamus-pituitary-adrenal (HPA) axis by Marelli et al. (2010). This circulation binds to the cytosolic glucocorticoid receptor (NR3C1) in the cytoplasm. At the nucleus, the receptor can regulate gene transcription by directly binding to glucocorticoid response element (GRE) which has a position at the promoter of the target gene, for anti-inflammatory. Similarly, Marelli et al. (2010) found that there were significant differences between three Italian breeds, where the fast-growing type had the higher expression and...
the study suggested that it was due to the difference in coping ability among breed. In contrast, Rimoldi et al. (2015) found non-significant difference between fast-growing and slow-growing breed under acute heat stress and control group in response to NR3C1 gene.

In Figure 2, the trend indicates dramatic different expression within breeds and time of blood sampling. Tanaosree and Khai Mook Esarn KKU50 demonstrated higher expression than the native line with the up-regulation of its activity. Moreover, the trend shows the highest expression at the week 12, and they were quite a different expression. However, following the statistical test, there was no significant different to the interaction between breeds and time of sampling (P>0.05).

![NR3C1 gene](image)

**Figure 2** Log$_2$ relative normalized expression (2$^{-\Delta\Delta C_{t}}$) in expression of Glucocorticoid receptor (NR3C1) gene relative to the control gene (18S-RNA) due to individual ability in three different breeds Chee, Khai Mook Esarn and Tanaosree under different time of blood collection at week 4, week 8 and week 12, where the combination were significantly different at (P<0.05).

Starting from the 8th week, Khai Mook Esarn KKU50 chickens had lower body weight than Tanaosree chickens. Particularly at the 12th week, all breeds demonstrated the highest expression and quite varied each other. This the evident to indicate that the faster-growing breed also expressed higher in NR3C1 gene similarly to Soleimani et al. (2008) who demonstrate that the selection for growth performance reduced responsiveness of cortisol to stress. The result finding in the current study affirms the study of Gihan et al. (2009) and Geraert et al. (1993) where they illustrated that the fast-growing period expressed higher stress.

**Conclusions**

In conclusion, we report the absolute expression level of NR3C1 gene in 3 Thai chickens under the same management. The NR3C1 gene expression may imply the response of pituitary regulation to glucocorticoid secretion under stress condition of chickens. The slower-growing breed (Chee KKU12) had higher coping ability than faster-growing breeds (Khai Mook Esarn KKU50 and Tanaosree chickens). Hence, the NR3C1 gene expression varied in breed would be potential information for assessing birds for growth performance and coping ability under the opening house system, would be needed future verification.
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References


