

# Administration of Extract *Salix tetrasperma* Combined with Extract of Turmeric and Neem to Improve Eggs Quality of Chicken Reared under Heat Stress

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## ABSTRAK

Sugito, Hambal M, Isa M, Nurliana, Delima M. 2015. Pemberian ekstrak *Salix tetrasperma* kombinasi dengan ekstrak kunyit dan mimba untuk peningkatan kualitas telur ayam yang dipelihara pada suasana stres panas. JITV 20(3): 193-199. DOI: <http://dx.doi.org/10.14334/jitv.v20i3.1186>

Kualitas telur akan menurun apabila ayam mengalami stres panas. Pemberian beberapa jenis ekstrak tanaman dilaporkan dapat mengurangi dampak tersebut. Tujuan penelitian ini adalah untuk mengetahui efek pemberian ekstrak *Salix tetrasperma* dikombinasikan dengan ekstrak kunyit dan mimba untuk meningkatkan kualitas dan produktivitas ayam petelur dalam kondisi stres panas. Penelitian ini menggunakan 60 ekor ayam petelur strain Isa Brown periode layer berumur 6 bulan. Pemeliharaan dilakukan pada kandang kawat individu. Pemberian pakan dan minum secara ad libitum. Pelaksanaan penelitian menggunakan metode rancangan acak lengkap dengan 5 perlakuan dan masing-masing perlakuan terdiri atas 12 ulangan. Sebagai kontrol negatif tanpa diberi suplemen antistres (KN) dan kontrol positif diberi suplemen antistres komersial (KP). Formulasi dosis ekstrak yang diberikan adalah *S. tetrasperma* 1.000 mg/l air minum (EJ), *S. tetrasperma* 1.000 mg/l + kunyit 250 mg/l + mimba 250 mg/l (EJ+K1), dan *S. tetrasperma* 1.000 mg/l + kunyit 500 mg/l + mimba 500 mg/l air minum (EJ+K2). Ayam dipelihara dalam suasana stres panas selama 5 jam sehari pada kisaran suhu  $34,0 \pm 1,5^{\circ}\text{C}$ . Pemberian suplemen dilakukan dengan cara dilarutkan dalam air minum dan diberikan selama 30 hari pada pagi sampai siang hari. Hasil penelitian menunjukkan bahwa pemberian ekstrak *S. tetrasperma* secara tunggal ataupun dikombinasi dengan ekstrak kunyit dan mimba mempengaruhi ( $P < 0,05$ ) tebal kerabang telur, tetapi tidak mempengaruhi warna kuning telur, tinggi albumin, berat telur, dan nilai Haugh Unit (HU). Pemberian ekstrak *S. tetrasperma* 1.000 mg/l air minum dapat digunakan untuk meningkatkan kualitas telur pada ayam yang menderita stres panas.

**Kata Kunci:** Kualitas Telur, *Salix tetrasperma*, Kunyit, Mimba, Stres Panas

## ABSTRACT

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Quality of eggs might decrease when hens under heat stress. A further study found that a specific plant extracts could reduce the impacts of heat stress. The aim of this study was to determine effects of *Salix tetrasperma* plant extract in combination with extract of turmeric and neem to improve egg quality and productivity of laying hens under heat stress. Sixty laying hens strain Isa Brown of 6 months old were used and reared in individual cages. The feed and drinking water were supplied ad libitum. This study was conducted in a completely randomized design with five treatments (two controls and three treatments) and each treatment consisted of 12 replication. Treatment consisted of with (KP) and without (KN) commercial anti-stress supplement. Formulations of extract were *S. tetrasperma* 1.000 mg / l water (EJ), *S. tetrasperma* 1.000 mg / l + Turmeric 250 mg / l + neem 250 mg / l (EJ+K1), and *S. tetrasperma* 1.000 mg / l + Turmeric 500 mg / l + neem 500 mg / l drinking water (EJ+K2). The hens were exposed to heat stress for 5 hours per day at a temperature range of  $34.0 \pm 1.0^{\circ}\text{C}$ . Supplements were dissolved in drinking water and were given for 30 days in the morning and noon. Results showed that a single extract of *S. tetrasperma* or the combination of an extract of turmeric and neem were significantly increased thickness of eggshell ( $P < 0.05$ ), but did not affect color of egg yolk, height albumin, egg weight, and HU value. Extract of *S. tetrasperma* combined with turmeric and neem extract dissolved in drinking water for 30 days in laying hens reared under heat stress could not improved quality of the eggs, but may increase thickness of the egg shell and cause decreased water consumption.

**Key Words:** Egg Quality, *Salix tetrasperma*, Turmeric, Neem, Heat Stress

## INTRODUCTION

Daily environment temperature in several areas in Aceh Province is relatively high. During the day in the dry season, temperature is around 31-35°C. Ahmadi & Rahimi (2011) said that temperature and humidity in an area became a critical factor affecting laying hen production. Laying hens kept out of their thermoneutral condition experience physiology change and decreasing their egg production and quality. According to Talukder et al. (2010) and Feizi et al. (2012), optimum environment temperature for laying hen productivity was around 15-27°C with relative humidity around 60-70%. Higher temperature and humidity than 27°C and 70% respectively causes heat stress. Heat stress decreases body weight, egg production, consumption, egg weight, and egg tickness of laying hen.

Herbs used may decrease heat stress impact in laying hen. Previous research showed that administration of *Salix tetrasperma* Roxb plant extract in broiler chicken may decrease heat stress impact (Sugito et al. 2006). The last research result showed than administration of *Salix tetrasperma*, both as a single or combined with extract of tumeric and neem may be used to decrease heat stress impact and did not affect health status of laying hen (Sugito et al. 2014).

Attempt to improve potential of *S. tetrasperma* extract by formulating it with other plant may decrease heat stress impact. Tumeric and neem were chosen supported by fact that administration of turmeric or neem may decrease negative impact of heat stress in chicken, as reported by Riasi et al. (2012) that supplementation with turmeric meal by 0.5-2 g/kg diets may improve egg quality and productivity. Nadia et al. (2008) also reported that administration of turmeric meal of 0.5% of diet may be used as antioxidant in laying hen. Moreover, Esonu et al. (2007) reported that administration of neem up to 15% may increase egg production and yolk color.

Dey et al. (2011) said that administration of diet contained neem meal by 10-15g/kg in laying hen may

increase albumen index and had positive quadratic impact in Haugh Unit (HU). Decrease of heat stress impact is related to bioactive compound in plants. The main bioactive compound of the *S. tetrasperma* is salicylic acid, curcumin in the turmeric and nimbidin in the neem plant and those derivatives. By formulating those 3 plants, it is be expected that afication of the bioactive compound will has better synergy impact to decrease heat stress impact in laying hen.

This study was aimed to determine effect of administration of *S. tetrasperma* extract single or combined with extract of turmeric and neem to improve quality and egg productivity of laying hen experienced heat stress.

## MATERIALS AND METHODS

### Laying hens

Sixty 6 months old laying hens strain Isa Brown were used in this study. Those hens were kept in individual wire cage. Diet and drinking water were given ad libitum. Before treated, the hens were adapted for 1 week. This study was done based on completely randomized design with 5 treatments and 12 replications for each treatment.

### Treatments

Treatments given was showed in Table 1. Anti-stress supplement was dissolved in the drinking water and given for 30 days in the morning and noon. Administration and replacement of diets was done in the noon. Diet fed was commercial feed code 234-1.

Hens were kept in cage temperature was increased up to 32-34°C for 5 hours/day for 30 days to inflict heat stress condition. Temperature and humidity were controlled using digital thermometer. To get the temperature range, eight 100 watt bulbs were lighted. Fan was used to remove heat. Lighting used 20 watt neon lamps lighted for 10 hours/day.

**Table 1.** Treatment of supplement administered to laying hens with extract of *S. tetrasperma* leaf, turmeric rizhome, and neem leaf

Extract Material	Control (-) (KN)	Control (+) (KP)	Extract of <i>S. tetrasperma</i> (EJ)	Extract of <i>S. tetrasperma</i> + Combination 1 (EJ+K1)	Extract of <i>S. tetrasperma</i> + Combination 2 (EJ+K2)
Commercial anti-stress	-	5 mg/lit	-	-	-
<i>S. tetrasperma</i>	-	-	1000	1000	1000
Turmeric	-	-	-	250	500
Neem	-	-	-	250	500

### **Extraction of *S. tetrasperma* leaf, turmeric rhizome, and neem leaf**

Extraction of *S. tetrasperma* leaf, turmeric rhizome, and neem leaf were conducted by maceration method. *S. tetrasperma* leaf, turmeric rhizome, and neem leaf were cleaned, dried, and powdered before it was macerated. Each simple material was macerated 2 times by ethanol 70%. Filtrate obtained was concentrated using rotary evaporator to be condensed extract. The condensed extracts were made with each concentration as in Table 1 processed to be effervescent blend with material as follow: citric acid, tartaric acid, sodium bicarbonate, binder, and emulsifier of carboxymethyl-cellulose (CMC). Its administration was dissolved in the drinking water.

### **Parameters**

Variables measured were egg production for 30 days, diet consumption, consumption of water added by supplement, yolk color, albumen height, egg weight, Haugh Unit (HU) value, and eggshell thickness. Assessment of yolk color, albumen height, egg weight, and HU value were done using Egg Analyzer (Made in Japan). Egg Shell Thickness Gauge (Orka Technology Ltd) was used to measure eggshell thickness.

### **Statistical analysis**

Variables observed was analyzed by ANOVA with significant level by 5%. Significant effect from the treatment was further tested by Duncan multiple range test.

## **RESULTS AND DISCUSSION**

### **Environment condition while research**

During this study, temperature outside and inside of cage and daily humidity was presented in Figure 1. The highest temperature outside of cage at around 12.00-14.00 was around 35.6-36.0°C. Average temperature and humidity inside the cage were around 33.7-34.1°C and 58.4-50.1% respectively. Those temperature and humidity were in danger category (THI range= 83-86) shown by behavior change of whole hens such as panting. According to Muchacka et al. (2012), in chicken experiencing heat stress, behavior changes will be seen. Those changes are effort to eliminate or

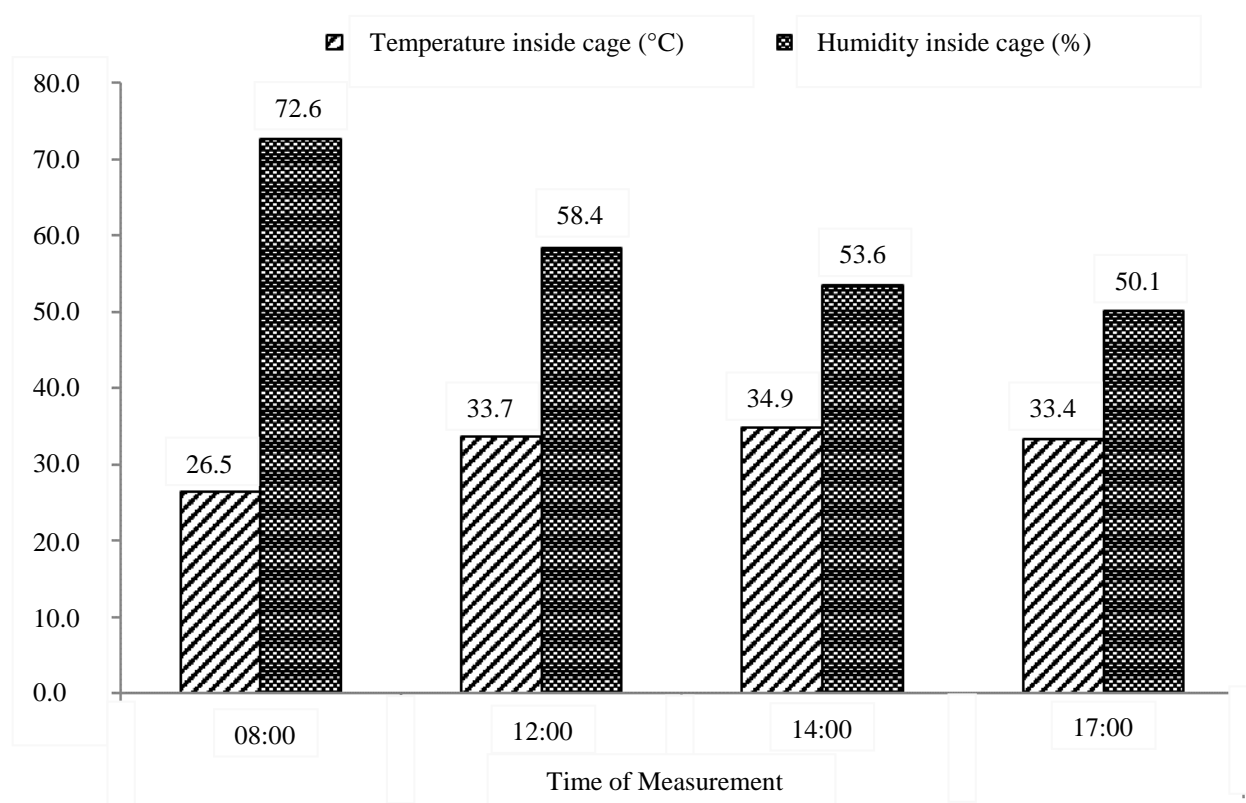
decrease heat stress in their body. Clinically, in chicken experienced heat stress experience increase in respiratory (panting). Panting indicated that chicken used in this study suffered heat stress.

### **Hens performance and egg quality**

Average egg production, diet and water consumption, and ratio of feed conversion of laying hens during this study were presented in Table 2. Administration of *S. tetrasperma* and its combination was not significantly ( $P>0.05$ ) affect egg production. In the EJ+K1 was a treatment with highest egg production (reaching 99.4%) with the lowest RKP value compared to the other treatments. Addition of *S. tetrasperma* extract combined with extract of turmeric and neem by each became 500 mg/l of drink water in fact may not affect egg production, diet consumption, and ratio of feed conversion, but significantly ( $p<0.01$ ) affecting water consumption. Result of comparative test showed that administration of *S. tetrasperma* extract combined with extract of turmeric and neem may decrease ( $P<0.05$ ) water consumption. The most water consumption in hens treated by EJ and the lowest one was in EJ+K2 treatment (Table 2).

Impact of heat stress to egg production, yolk color value, albumen height, HU value, and RKP value in hens has not been seen, both of Negative Control (Table 1 and 2) or addition of *S. tetrasperma* extract and its combination. This is in contrast to Mashaly et al. (2004) and Kilic & Simsek (2013) reported that heat stress in laying hens lead to decrease of production and quality of egg and diet consumption. Allahverdi et al. (2013) said that heat stress in chicken caused increase of free radical compound, disturbance of acid-base balance, and calcium metabolic disturbance. This physiology change affected production and quality of egg.

Administration of *S. tetrasperma* extract as single (EJ treatment) did not cause decrease of water consumption, instead the number of water consumed was more than those of EJ+K1 and EJ+K2. This was suspected that administration of *S. tetrasperma* did not affect taste of the drinking water. In contrast to that, administration of *S. tetrasperma* combined with extract of turmeric and neem caused decrease of water consumption. That decrease in those EJ+K1 and EJ+K2 treatments was suspected due to the water was relatively bitter than those KN, KP, and EJ treatments. Kudo et al. (2010) said that laying hen was a sensitive bird to bitter taste. Bitter taste in the diet and drinking water caused decrease in consumption.



**Figure 1.** Average daily temperature (°C) and humidity (%) for 30 days inside cage during this study

Average yolk color, albumin height, egg weight, HU value, and eggshell thickness were shown in Table 3. Statistic test result showed that administration of *S. tetrasperma* or its combination did not affect yolk color, albumen height, egg weight, and HU value, but significantly ( $P < 0.01$ ) increased eggshell thickness. The EJ treatment tend to increase eggshell thickness. Increasing dose of turmeric and neem extract (EJ+K2 treatment) seemed did not decrease eggshell thickness. In the chicken experienced heat stress (KN treatment) may experience decrease of eggshell thickness.

Increasing of eggshell thickness of hens fed by EJ, EJ+K1, and EJK2 was suspected related to free radical forming when the hens suffering heat stress. The increasing was very high compared to that when hens in the comfort zone (24-26°C). Critical impact free radical to cell metabolism was its involvement in lipid peroxide reaction and this condition may cause damage or death of cell (Fotina et al. 2013). This was proved by administration of *S. tetrasperma* leaf extract combined with extract of turmeric rhizome and neem leaf may increase the eggshell thickness (Table 1 and 2) compared to those of EJ and KP treatments. That increasing was an effect of activity of compound therein. Bioactive compound in those extracts were suspected playing important role to decrease heat stress

impact. The most compounds in those extracts were as antioxidant (Al-Harathi 2014). Result of studies showed that there were several bioactive compounds played role as antioxidant in extract of *S. tetrasperma* (Kahkonen et al. 1999; Khayyal et al. 2005; El-Wakil et al. 2015), turmeric rhizome (Gupta et al. 2010; Mohana & Fadma 2014), and neem leaf (Kumar et al. 2010).

Beside as antioxidant, several bioactive compounds in plant extract such as flavonoid compound may prevent steroid hormone activity (Zand et al. 2000). Physiology respond for body homeostatis by releasing stress hormones such as corticosteroid occurred when chicken experienced heat stress. Corticosteroid hormone disrupted body metabolism and then aggravated chicken body physiology. It was also suspected that those bioactive compounds in those *S. tetrasperma*, turmeric, and neem may disturb activity of adrenal steroidogenic enzymes and receptor sensitivity of glucocorticoid hormones (Schloms & Swart 2014; Al-Daraji 2012), so that secretion of this hormone was disrupted. This caused heat stress do not affect chicken body physiology.

Role of the extract of *S. tetrasperma*, turmeric, and neem to increase eggshell thickness was suspected through calcium and phosphor metabolism. In chicken

**Table 2.** Percentage of egg production, average ( $\pm$ SD) diet consumption, drinking water, and feed conversion ratio value of laying hen

Treatment	Egg Production (%)	Diet Consumption (g/hari/ekor)	Consumption of Drinking Water (ml/hari/ekor)	Feed Conversion ratio
KN	96.1 (346)	104.1 $\pm$ 8.2	177.6 $\pm$ 24.8 <sup>ac</sup>	2.01 $\pm$ 0.60
KP	98.1 (353)	101.4 $\pm$ 9.2	191.2 $\pm$ 44.9 <sup>a</sup>	1.81 $\pm$ 0.12
EJ	98.6 (355)	102.3 $\pm$ 9.3	195.5 $\pm$ 47.0 <sup>a</sup>	1.82 $\pm$ 0.12
EJ+K1	99.4 (358)	104.7 $\pm$ 5.5	155.5 $\pm$ 27.1 <sup>bc</sup>	1.73 $\pm$ 0.11
EJ+K2	98.3 (354)	102.3 $\pm$ 5.8	139.9 $\pm$ 39.3 <sup>b</sup>	1.83 $\pm$ 0.16

KN = negative control treatment without anti-stress supplement

KP = positive control treatment with commercial anti-stress supplement

EJ = *S. tetrasperma* extract treatment by 1000 mg/l of drinking water dose

EJ+K1 = *S. tetrasperma* extract 1000 mg/l + turmeric 250 mg/l + neem 250 mg/l

EJ+K2 = *S. tetrasperma* extract 1000 mg/l + turmeric 500 mg/l + neem 500 mg/l of drinking water

**Table 3.** Average ( $\pm$ SD) of yolk color, albumen height, egg weight, HU value, and eggshell thickness

Treatment	Yolk Color	Albumen Height	Egg Weight (gr)	HU	Eggshell Thickness (mm)
KN	9.70 $\pm$ 1.06	5.63 $\pm$ 1.68	56.07 $\pm$ 3.88	73.43 $\pm$ 15.73	0.370 $\pm$ 0.017 <sup>a</sup>
KP	9.91 $\pm$ 1.04	6.02 $\pm$ 1.45	55.67 $\pm$ 4.47	77.66 $\pm$ 9.79	0.368 $\pm$ 0.020 <sup>a</sup>
EJ	10.09 $\pm$ 1.22	6.06 $\pm$ 1.05	56.16 $\pm$ 4.46	78.20 $\pm$ 6.84	0.405 $\pm$ 0.026 <sup>b</sup>
EJ+K1	10.18 $\pm$ 0.98	6.57 $\pm$ 0.99	57.79 $\pm$ 3.23	81.23 $\pm$ 6.50	0.400 $\pm$ 0.032 <sup>b</sup>
EJ+K2	9.75 $\pm$ 1.14	6.13 $\pm$ 1.64	53.25 $\pm$ 4.10	78.04 $\pm$ 16.64	0.399 $\pm$ 0.021 <sup>b</sup>

experienced heat stress, calcium level in blood was decreased. This caused hot eggshell formation disrupted (Balnave 1998). Eggshell formation needs sufficient calcium and carbonate in the uterus. Before calcification of eggshell, calcium was not stored in the uterus, but in blood plasma in the form of calcium ion. Mineralization of calcium carbonate was done in the uterus (Ahmadi & Rahimi 2011). Radwan et al. (2008) said that several bioactive compound of plant extract may improve micro environment condition in the chicken's uterus, so that egg mineralization may occur more perfectly and the eggshell was thicker. Activity of estrogen hormone was also suspected may increase eggshell thickness. Lukic et al. (2011) said that involvement of estrogen hormone in the calcium metabolism of eggshell was very important. Furthermore, Nakari (2005) and Noppe et al. (2008) reported that in plant extract there was phytosterol compound which may serve as steroid hormones. Sugito (2007) reported that *S. tetrasperma* plant contained steroid compound reaching 10.08%, while El-Shazly et al. (2012) has identified several phytosterols in salix plant, namely: beta-sitosterol acetate, friedelin, 3beta-friedelinol, beta-amyrin, beta-sitosterol, beta-sitosterol-O-glucoside, palmitic acid, catechol, and

tremulacin. Turmeric extract contained several phytosterol (Sawant & Godghate 2013).

## CONCLUSION

Administration of *S. tetrasperma* extract combined with turmeric extract and neem dissolved in drinking water for 30 days in laying hens kept in heat stress did not increase egg quality, but may increase eggshell thickness and caused decreased of water consumption.

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