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Effect of Mangosteen (*Garcinia Mangostana*) Pericarp Extract in Reducing the Heat Stress of Laying Quails

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Abstract | The study was investigated the effects of mangosteen (*Garcinia mangostana*) pericarp extract (MCE) dissolved in drinking wateron the heat stress (hot season) of laying quails. A total of 700 laying quails with average initial egg production of 82.38% ± 0.98 were randomly assigned to one of four treatment groups. Each group had seven replications of 25 quails for each. The treatment groups included a control group that did not receive mangosteen pericarp crude extract (MCE) supplementation and three groups that received dilution ratios of MCE to water of 1:5 (MCE5), 1:10 (MCE10) and 1:15 (MCE15), respectively. The quails were consumed the different MCE dilutions during summer for 16 weeks. The temperature was ranged from 35 °C to 37 °C. The experimental quails were exposed to chronic heat stress for four hours daily under an open-housed system. The result showed that the feed conversion ratio, yolk color, total cholesterol, and aspartate aminotransferase were unaffected by the MCE treatments ($P > 0.05$). However, feed consumption, egg production, egg weight, and egg mass were significantly increased by the addition of MCE ($P \leq 0.05$). Linear improvements in Haugh unit ($P= 0.014$), specific gravity ($P= 0.089$) and eggshell components ($P= 0.033$) were detected with increasing dilutions of MCE. Heterophil-to-lymphocyte ratios (H/L ratio) also tended to be lower in the quails that received MCE treatment ($P= 0.071$). Low-density lipoproteins and glucose concentrations decreased, whereas an increase in high-density lipoprotein was detected with the addition of MCE ($P \leq 0.05$) increased. We furthermore the fecal microbial populations such as *Salmonella enteritidis*, *Lactobacillus* spp. and *Escherichia coli* were influenced by MCE treatment in the laying quails ($P \leq 0.05$). In conclusion, we found that the addition of MCE to drinking water could be an alternative approach to reduce the heat stress effect in laying quails.

Keywords | Gut microbiota, Heat stress, Laying quail, Mangosteen pericarp

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INTRODUCTION

Heat stress (HS) is a critical issue in the poultry industry, especially in hot regions, because it impairs growth performance, feed consumption, immunity and liability percentage. It has been established that an increase in temperature to 34 °C daily causes detrimental intestinal injury in broiler chickens by activation of the hypothalamic-pituitary-adrenal (HPA) axis and corticosterone concentrations (Garriga et al., 2006; Quinteiro-Filho et al., 2010; Sahin et al., 2017). High circulating levels of corticosterone are known to induce immunosuppression and susceptibility to infectious disease (Honda et al., 2015). In laying birds, daily exposure to high temperature (42 °C ± 3 °C) for 12 h is associated with decreases in ovarian function, egg production, egg size, ovarian weight, and the number of large follicles due to inhibition of the secretion of steroidogenic enzymes (Rozenboim et al., 2007). Studies have also found that acid-base regulation may be impaired in birds exposed to heat stress, resulting in poor eggshell quality (Lin et al., 2004). Numerous methods have been applied in drinking water to eliminate heat stress and im-

prove laying performance (Ahmad et al., 2008). However, little is known about the effects of medicinal plant extracts (Alipour et al., 2015; Diaz-Sanchez et al., 2015). Therefore, it is challenging for poultry producers to find new strategies to solve the problem of environmental stressors.

Garcinia mangostana (mangosteen) is a tropical evergreen tree, common in Indonesia, Myanmar, Sri Lanka, the Philippines and Thailand (Pedraza-Chaverri et al., 2008). The genus *Garcinia* contains a variety of phenolic compounds, including tannins, anthocyanins, and xanthones (Zadernowski et al., 2009). Recently, it has been found that the pericarp of *G. mangostana* is rich in 9-hydroxycalabaxanthone, parvifolixanthone, α-mangostin and rubraxanthone (Mohamed et al., 2014). These compounds have various biological effects, such as antibacterial (Suksamran et al., 2003), anti-inflammatory (Chen et al., 2008), anticancer (Yu et al., 2009), and antioxidant properties (Mohamed et al., 2014; Thong et al., 2015). A laying trial found that the inclusion of 1 g mangosteen pericarp per kilogram of diet resulted in improved eggshell thickness and decreases in yolk cholesterol and blood triglyceride concentrations (Rusli et al., 2015). However, no data are available on the effects of adding mangosteen crude extract (MCE) to the drinking water of laying quails exposed to high ambient temperatures. Consequently, an experiment was carried out to investigate the effects of adding extract of mangosteen pericarp to the drinking water of laying quails on productive performance, egg quality, heterophil-to-lymphocyte (H:L) ratio, blood metabolites and gut microbiota during summer.

MATERIALS AND METHODS

Preparation of Mangosteen Pericarp Extract

Approximately 12 years of mangosteen pericarp by-product was collected from Chatuchak market (Bangkok, Thailand). The mangosteen residue was washed with tap water to eliminate unwanted materials and directly dried in an oven at 100 °C for 96 h. After complete cooling to room temperature, the samples were ground, and an extract from the pericarp was made following the method of Tjahjani et al. (2014). The extraction solvent was then separated and filtered with Whatman No. 1 filter paper (Sigma-Aldrich Inc., Darmstadt, Germany). The extracted layer was further evaporated using rotary evaporator (Scientific Industry and Trade Co., Ltd., Zhengzhou, China). The yield of 500 g dried weight of mangosteen pericarp extract powder was 11.48% (w/w).

Hosing Condition and Experimental Diet

Seven hundred laying quails at 90 days of age were assigned at random to one of four treatments based on their average egg production (82.38%). Each group consisted of seven replicates with 25 quails. The treatments included several MCE dilutions with tap water as follows: treatment without MCE (CON), MCE5, MCE10 and MCE15, which were MCE in tap water at ratios of 1:5, 1:10, and 1:15, respectively. All birds were given the different MCE dilutions from 90 to 202 days of age. The experiment was conducted from April to July (16 weeks) in Ang Thong province in central Thailand. The experimental laying quails were raised in an open-housed system in which the temperature ranged from 29 °C to 33 °C in the morning (from 9 to 12 am) and 35 °C to 37 °C in the afternoon (from 1 to 5 pm). The birds were exposed to artificial light daily from 04:00 to 20:00 (18L: 6D). Feed and water were given twice daily at 08:00 and 15:00 to ensure that all experimental birds had free access to feed and water throughout the course of the study.

Table 1: Nutrient composition of experimental diet (% as fed basis)

Ingredient (%)	Amount
Corn	52.71
Soybean meal-45%	10.00
Corn gluten meal	12.89
Rice bran	1.43
Cassava meal	20.00
Rice bran oil	0.87
L-lysine sulfate (78%)	0.18
DL-methionine (99%)	0.10
L-tryptophan	0.02
Dicalcium phosphate	1.44
Salt	0.35
Limestone	0.01
Calculated value	
Metabolizable energy, kcal/kg	2,900
Crude protein (%)	24.00
Lysine (%)	1.20
Methionine	0.54
Methionine + cysteine	0.84
Calcium (%)	0.80
Available phosphorus (%)	0.30
Analyzed composition	
Crude fat (%)	4.91
Crude protein (%)	23.84
Ash (%)	9.82
Moisture (%)	4.26

[1]Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 1, 600 IU; vitamin E, 34 IU; d-biotin, 64g; riboflavin, 3.4 mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B_{12}, 12g; vitamin K, 2.4mg, Se, 0.1mg; I, 0.32 mg; Mn, 25.2 mg; $CuSO_4$, 53.9 mg; Fe, 127.3mg; Zn, 83.46 mg; Co, 0.28 mg.

The experimental diets were similar in all treatments and were formulated to meet or exceed the standard nutrient requirement of laying quails in a peak period (Table 1) (NRC, 1994). All experimental care and handling of laying quails were in accordance with the animal committee approved by the National Research of Thailand (Bangkok, Thailand).

Egg Production and Feed Efficiency

The eggs in each cage were counted, collected and weighed daily at 15:00. These data were used to calculate hen-day egg production and average egg weight. Egg mass was calculated as (hen-day egg production x average egg weight)/100. The residue and added feed were collected at the 0, 4,8,12, and 16 weeks of age, and shown as a cumulative data. These data were used to calculate the feed conversion ratio **(**FCR**)** and feed consumption. The number of dead laying quails in each replicate was used to adjust feed consumption. The number of cracked eggs, including those with hairline cracks, pinhole cracks and body cracks, was recorded and summarised as the percentage of damaged eggs. All criteria for laying performance and feed efficiency were included in the cumulative data.

Egg Quality

Egg quality traits including Haugh unit, specific gravity, eggshell component, eggshell thickness and yolk color score were measured every 4 weeks, using 42 eggs per treatment (n = 168 samples) with an average egg weight of 10.34 ± 1.13 g. Whole selected eggs were used to determine egg specific gravity using concentrations of NaCl ranging from 1.070 to 1.090. The eggshell composition was calculated by subtracting the albumen plus yolk weight from the whole egg weight. Yolk color was scored using a Roche color fan (Technical Services and Supplies, York, UK). The albumen height was measured in three different positions, and its value was used to calculate albumen freshness using the formula of Eisen et al. (1962) [Haugh unit = 100 × log (H + 7.57 – 1.7 × $W^{0.37}$)], where H is the albumen height (mm) and W is the egg weight (g) (Technical Services and Supplies, York, UK).The eggshell thickness was measured after removing adhering albumen with tap water and drying for 72 h at room temperature. The eggshell measurements were taken three times around the equator of the egg using a digital micrometer (Mitutoyo Corporation, Kanagawa, Japan), and the average value for each egg was recorded.

Blood Collection and Analyses

Twenty birds were randomly selected before conducting the experiment and a total of 84 birds (three birds per replication) were selected at the end of the experiment. Blood collection was performed from 09:00 to 10:00 using sterilized needles and syringes via a jugular vein, and the procedure was completed within a minute to avoid handling stress. Blood samples were directly transferred into 3-mL ethylenediaminetetraacetic acid (EDTA) anticoagulant tube (VacutestKima Inc., Italy) and placed at room temperature for 2 hours to separate sera. After 2 h, blood was centrifuged at 3,000 rpm for 10 minutes. Sera samples were transferred into a 1.5-mL tube using a pipette for measurement of H/L ratios. Briefly, one drop of blood was smeared on a glass slide, and each drop was tested in triplicate. The slides were dried at room temperature before immersion in 95% methyl alcohol solution. Each smeared slide was post-fixed every 20 min a total of 5 times. The slides were manually stained using Wright-Giemsa's method following the manufacturer's guidelines (method WSGD-128, Sigma Chemical, St. Louis, MO). The heterophils and lymphocytes on 100 slides were immediately counted by the same trainer using a light microscope (Olympus American, Center Valley, PA). The remaining sera was used for analyses of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (AST), and glucose concentrations using commercial test kits (Zhongsheng Biochemical Co., Ltd., Beijing, China). All samples were run in triplicate using an automatic biochemical analyser (ADVIA 120, Bayer, Tarrytown, NY) according to the manufacturer's instructions, and analysis was performed in the same conditions to avoid assay variations.

Microbial Enumeration

Three laying quails in each replication were randomly selected at the end of the feeding trial for the collection of faecal samples. The samples (approximately 1 g) were serially diluted 10-fold with 0.9% NaCl and homogenised for 5 min by vortex mixing (Scientific Industries, Inc., New York). Microbial enumeration was then performed on agar plates (Difco Laboratories, Inc., Detroit, MI).After incubation for 48 h at 30 ˚C, the occurrence of *S.enteritidis* on *S.enteritidis* agar, *Lactobacillus* spp. on *Lactobacillus* MRS broth and *E. coli* on MacConkey agar was recorded. All colonies of each microorganism were counted using a colony counter (Selby, Model SCC100, Sydney, Australia). Each value was recorded as base-10 log cfu/gram of faecal digest.

Yolk Collection and Analysis

Eighty-four eggs (three eggs for each replication) were randomly chosen at 15:00 on week 8 and the last day of the experiment for yolk cholesterol and triglyceride assays. The egg yolks were directly separated from egg whites and kept at -20 ˚C for further laboratory analyses. The concentration of yolk cholesterol was detected by gas chromatography according to the procedure of Will and Greenfield (1984), whereas the concentration of yolk triglyceride was detected by commercial test kit, similar to the blood analysis.

Table 2: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on egg production in laying quails throughout the entire period

Criteria	CON	MCE5	MCE10	MCE15	SEM[1]	*P* - value	
						Linear	Quadratic
Egg production (%)	78.51[b]	87.86[a]	88.27[a]	88.10[a]	1.356	0.010	0.054
Egg weight (g/egg)	10.68[b]	12.56[a]	12.19[a]	12.51[a]	0.278	0.025	0.029
Egg mass (g/day/bird)	8.38[b]	11.04[a]	10.76[a]	11.02[a]	0.336	0.003	0.011
FCR (g feed/g egg)	2.92	2.60	2.63	2.46	0.101	0.109	0.170
Feed consumption (g/day/bird)	24.45[b]	28.70[a]	28.39[a]	27.02[ab]	0.970	0.193	0.254
Damaged eggs (%)[2]	9.92[a]	4.60[b]	5.31[b]	5.63[b]	0688	0.043	0.061

FCR = feed conversion ratio, CON = control treatment with no addition of mangosteen pericarp extract to drinking water, MCE5, MCE10 and MCE15 = mangosteen pericarp extract addition to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.05$).

Table 3: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on interior egg quality of laying quails throughout the entire period[1]

Criteria	CON	MCE5	MCE10	MCE15	SEM[2]	*P* - value	
						Linear	Quadratic
Haugh unit	81.93[b]	88.69[a]	87.81[a]	87.92[a]	0.916	0.014	0.027
Specific gravity (wt/vol)	1.070[b]	1.080[a]	1.076[ab]	1.078[ab]	0.001	0.089	0.045
Eggshell component (%)	7.76	8.26	8.51	8.44	0.006	0.033	0.198
Eggshell thickness (mm)	0.243[b]	0.279[ab]	0.286[a]	0.282[a]	0.186	0.159	0.546
Yolk color score	4.50	4.67	4.83	4.83	0.141	0.417	0..793

CON = control treatment with no addition of mangosteen pericarp extract to drinking water; MCE5, MCE10, and MCE15 = mangosteen pericarp extract added to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]Values are presented as mean of 42 egg quails per treatment (n = 168 samples).
[2]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.05$).

Table 4: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on heterophil-to-lymphocyte ratio in laying quails[1]

Criteria	CON	MCE5	MCE10	MCE15	SEM[2]	*P* - value	
						Linear	Quadratic
Heterophils							
Initial	27.12	27.12	27.12	27.12	-	-	-
16 weeks	44.33	37.67	39.33	36.67	2.460	0.423	0.432
Lymphocytes							
Initial	53.46	53.46	53.46	53.46	-	-	-
16 weeks	36.00	41.33	37.00	41.67	1.883	0.360	0.131
H/L ratios							
Initial	0.55	0.55	0.55	0.55	-	-	-
16 weeks	1.21	0.93	1.03	0.89	0.051	0.071	0.056

H:L ratios = heterophil-to-lymphocyte ratios; CON = control treatment with no addition of mangosteen pericarp extract to drinking water; MCE5, MCE10, and MCE15 = mangosteen pericarp extract addition to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]Values are presented as mean of 21 quails per treatment (n = 84 samples).
[2]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.01$).

Statistical Analysis

Laying performance, egg quality, blood stress indicators, metabolic profiles, yolk cholesterol, yolk triglyceride and gut microbiota were statistically analysed according to a

completely randomised design using the general linear model procedure (SAS, Cary, NC). Each cage comprised an experimental unit for the detection of growth performance, whilst the selected quails and eggs comprised the experimental units for the detection of blood immune responses, metabolic profiles, gut microbiota, and yolk cholesterol and triglyceride concentrations. Significant differences amongst treatments were declared by Duncan's new multiple range test. Furthermore, the linear and quadratic effects of increasing the ratio of MCE dilution were used to evaluate treatment effects using orthogonal polynomial contrasts. Significance values of $P<0.05$ were used for the experimental treatments and $P<0.05$ to $P<0.10$ were used to indicate a tendency.

RESULTS

Laying Performance

The egg production and feed efficiency of the laying quails given MCE in drinking water is being summarised in Table 2. The cumulative data on the feed conversion ratio did not indicate a significant effect of different dilutions of MCE ($P\leq0.05$). However, the inclusion of MCE in drinking water was associated with improvements in egg production, egg weight, egg mass and feed consumption and a lower percentage of damaged eggs when compared to the quails that received the control treatment ($P \leq 0.05$). Linear and quadratic effects were found for the dilution levels of MCE on egg production ($P = 0.010$ and $P = 0.054$, respectively), egg weight ($P = 0.025$ and $P = 0.029$, respectively), egg mass ($P = 0.003$ and $P = 0.011$, respectively) and damaged egg percentage ($P = 0.043$ and $P = 0.061$, respectively) as the dilution increased.

Egg Quality

Measurements of the egg quality of the laying quails after providing MCE in drinking water are shown in Table 3. Eggshell composition and yolk color score were unaffected by MCE dilution among treatments, but a linear effect on eggshell composition was observed for increasing MCE dilutions ($P = 0.033$). Improvements in the Haugh unit and eggshell specific gravity were observed in the quails in the MCE5 group compared to those in the CON group ($P\leq0.05$), which showed linear ($P = 0.014$ and $P = 0.089$, respectively) and quadratic ($P= 0.027$ and $P = 0.045$, respectively) effects for both criteria when MCE dilutions were increased. In addition, the MCE10 and MCE15 groups were associated with significantly greater eggshell thickness than the CON group ($P \leq 0.05$).

Leukocyte Count

The leukocyte counts of laying quails given MCE in drinking water are shown in Table 4. No significant differences were observed in the initial period. In addition, the dilution level of MCE had no significant effect on the numbers of heterophils and lymphocytes in laying quails after consuming MCE for 16 weeks. At the end of the experiment, however, a reduction tendency was seen in linear ($P = 0.071$) and quadratic ($P = 0.056$) responses regarding increasing MCE dilutions on the H:L ratios.

Blood Metabolites

The blood metabolites of laying quails after receiving MCE in drinking water are given in Table 5. The differences of total cholesterol and AST among experimental treatments were unaffected by the addition of MCE. However, the quails given MCE showed enhanced metabolic function in lipid profiles with increased HDL (a linear response, $P = 0.006$) and lower LDL (a quadratic effect, $P = 0.067$) concentrations. These criteria were significantly influenced by the increasing dilution of MCE. In addition, as the dilution of MCE increased, the glucose concentration decreased significantly from 294.67 to 243.83 mg/dL ($P = 0.001$).

Microbial Enumeration

The microbial counts in laying quails given different MCE dilutions for 16 weeks are given in Table 6. The results reveal reductions in *S.enteritidis* and *E. coli* populations in the cecum of the laying quails that consumed the MCEs compared to those that did not ($P \leq 0.05$). There were linear and quadratic effects on decreasing numbers of *S. enteritidis* ($P = 0.001$ and $P = 0.011$, respectively) and *E. coli* ($P = 0.011$ and $P = 0.042$, respectively), as the MCE dilution increased. Furthermore, the population of faecal *Lactobacillus* spp. was significantly increased in the MCE treatment groups compared to those in the CON group ($P \leq 0.05$), which can be seen in the linear effect with the increasing MCE dilution ($P = 0.012$).

Yolk Cholesterol and Triglyceride Concentrations

The yolk cholesterol and triglyceride concentrations of laying quails given MCE in drinking water for 16 weeks are summarised in Table 7. The inclusion of MCE in drinking water significantly reduced the yolk cholesterol concentration at 16 weeks compared to the controls ($P \leq 0.05$). It was reduced from 15.22 to 12.84 mg per gram of yolk at 16weeks with a linear ($P = 0.038$) and quadratic response ($P = 0.043$).Furthermore, the addition of MCE5 was associated with a positive reduction in the triglyceride concentration in yolk ($P \leq 0.05$) at week 16. This reduction was quadratically influenced by increasing dilution of MCE ($P = 0.024$).

DISCUSSION

Heat stress leads to economic losses in poultry production

Table 5: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on blood metabolites in laying quails[1]

Criteria	CON	MCE5	MCE10	MCE15	SEM[3]	*P* - value	
						Linear	Quadratic
TC (mg/dL)	129.39	118.67	120.40	122.73	3.170	0.519	0.507
HDL (mg/dL)	65.88[b]	86.83[a]	82.39[a]	83.17[a]	2.336	0.006	0.789
LDL(mg/dL)	24.09	21.46	22.72	21.97	0.487	0.202	0.067
AST (mg/dL)	423.83	428.36	431.80	437.24	18.266	0.824	0.909
Glucose (mg/dL)	294.67[a]	287.84[a]	259.50[b]	243.83[b]	5.168	0.001	0.129

CON = control treatment with no addition of mangosteen pericarp extract to drinking water; MCE5, MCE10, and MCE15= mangosteen pericarp extract added to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]TC = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AST = aspartate aminotransferase.
[2]Values are presented as mean of 21 quails per treatment (n = 84 samples).
[3]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.05$).

Table 6: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on microbial counts (log cfu/g of wet digesta) of laying quails[1]

Criteria	CON	MCE5	MCE10	MCE15	SEM[2]	*P* - value	
						Linear	Quadratic
Salmonella enteritidis	6.67[a]	4.38[b]	4.24[b]	4.34[b]	0.278	0.001	0.011
Lactobacillus spp.	5.22[b]	7.72[a]	7.68[a]	7.60[a]	0.327	0.011	0.042
Escherichia coli	7.11[a]	4.82[b]	4.44[b]	5.19[b]	0.306	0.012	0.128

CON = control treatment with no addition of mangosteen pericarp extract to drinking water; MCE5, MCE10, and MCE15 = mangosteen pericarp extract added to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]Values are presented as mean of 21 quails per treatment (n = 84 samples).
[2]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.05$).

Table 7: Effects of mangosteen (*Garcinia mangostana*) pericarp extract in drinking water on yolk cholesterol and triglyceride concentrations in laying quails at weeks 8 and 16[1]

Criteria	CON	MCE5	MCE10	MCE15	SEM[2]	*P* - value	
						Linear	Quadratic
Yolk cholesterol (mg/g yolk)							
8 weeks	14.13	12.56	12.87	12.59	0.373	0.255	0.174
16 weeks	15.22[a]	12.84[b]	13.28[b]	13.13[b]	0.325	0.038	0.043
Triglyceride (mg/g yolk)							
8 weeks	191.74	169.17	175.31	179.00	3.708	0.354	0.229
16 weeks	211.57[a]	180.83[b]	197.34[ab]	195.74[ab]	3.544	0.293	0.024

CON = control treatment with no addition of mangosteen pericarp extract to drinking water; MCE5, MCE10, and MCE15 = mangosteen pericarp extract added to drinking water at the ratio of 1:5, 1:10 and 1:15, respectively.
[1]Values are presented as mean of 21 eggs per treatment (n = 84 samples).
[2]Standard error of the means.
Linear effect by dilution level of mangosteen pericarp extract in drinking water ($P \leq 0.05$).

This experiment revealed that the addition of MCE to drinking water at different dilutions showed a positive association with increased laying performance and feed consumption of laying quails. This indicates that the inclusion of MCE in water can sustain the production of laying quails exposed to high temperatures. No negative influence on feed consumption or depressed laying production was found. The results are consistent with previous published data that showed that polyphenolic compounds from plants are effective defences against losses associated with laying quail fertility and egg production (El-Tarabany, 2016) via eliminating various physiological adaptations to heat stress. It should be stated that the laying quails were able to consume enough feed for their production needs. However, birds raised at high ambient temperature may display physiological changes associated with low feed

consumption (Song et al., 2014; Alagawany et al., 2017), which further decreases egg product. This is in agreement with the current findings.

This study also shows that eggshell parameters were positively influenced by MCE dilution. The improvement in the quality of eggshell may relate to the quails' ability to maintain homeostasis during exposure to a high ambient temperature. However, occurrences of poor eggshell quality, low specific gravity and eggshell components were observed in quails that received the control treatment; they may have been affected by a high susceptibility to respiratory alkalosis (Ebeid et al., 2012) and lower calcium and protein concentrations (Zhou et al., 1998). These detrimental effects are severe problems for laying chickens because they cause a lower blood concentration of carbon dioxide, which is used for the synthesis of bicarbonate (Borges et al., 2007) and less protein for egg synthesis (Zhou et al., 1998). Limitation of these substances negatively affects eggshell quality and Haugh unit. Although the effect of MCE in drinking water to alleviate heat stress has not been established, positive effects may be explained by the function of some active components in mangosteen pericarp .It has been found in many trials that the pericarp of mangosteen contains large amounts of polyphenolic compounds that play an important antioxidant role (Jung et al., 2006; Mohamed et al., 2014; Tjahjani et al., 2014). This might inhibit the production of reactive oxygen species before attacking macromolecules, resulting in the prevention of lipid peroxidation and protein degradation (Maqsood et al., 2015).This presents as poor quality albumen freshness, as indicated by Haugh unit measurement. However, the current study did not find significantly increased yolk color scores in the MCE-supplemented treatments. The lack of significant difference may be due to the method of extracting mangosteen pericarp to purify anthocyanin. It should be noted that the different dilutions of MCE in drinking water are non-toxic and could be an alternative approach to improving albumen freshness and eggshell quality.

Heterophil-to-lymphocyte ratio is commonly used as an indicator of stress in birds (Cotter, 2015). Previous reports showed that birds typically respond to heat stress via increased secretion of glucocorticoids (Gross and Siegel, 1983). This mechanism not only decreases macrophage activity but also responsively increases blood glucose concentration (Soleimani and Zulkifli, 2010).The defence mechanism for chronic stress has a greater impact on the H:L ratio than on corticosterone concentration. An increased number of heterophils has been shown to impair lymphocyte function and increase infectious disease and inflammation. This study found that quails in the MCE-supplemented groups tended towards a decreased H:L ratio (P = 0.071) and blood glucose concentration (P = 0.001). The reductions in these values might be associated with the alleviation of heat stress by MCE phytochemicals inhibiting the migration of heterophilgranulocytes from the marginated pool into the blood stream (Harmon, 1998). This mechanism benefits the immune cellular response by defending against the invasion of pathogens. Glucose is a key metabolic substance in animals and can be released in response to stress. It has been shown that birds commonly increase the glucose level in the bloodstream during stressful events. The effects of increasing stress-hormone secretion through activation of the HPA axis lead to an increase in the ratio of H:L. This result was found with the reduction of glucose concentration in the laying quails receiving MCE treatment, which might be rich in several antioxidant compounds. These components have a potential effect on free-radical scavenging activity. It was consequently altered lowering in glucose concentration by inhibiting glucocorticoids secretion. The decreasing H:L ratio and glucose concentration indicate an influence on immunity and alleviation of stress in laying quails.

The dilution amount of MCE had a sustained influence on gut microflora.Numerous studies have found an inhibitory effect of polyphenolic compounds obtained from mangosteen pericarp on pathogenic invasion. These findings are consistent with those of this study, in which MCE treatments reduced *S.enteritis* and *E. coli*. According to Chanarat et al. (1997), the presence of polysaccharides in mangosteen pericarp can induce the activity of phagocytic cells against *S. enteritidis*. Sundaram et al. (1983) also showed that active compounds from the mangosteen pericarp can kill *E. coli*. A polyphenol isolated from mangosteen also acts as a prebiotic, and it has been revealed earlier that this mechanism begins after the intake of food rich in high-polyphenol compounds (Hidalgo et al.,2012). In the current study, the laying birds that received MCE showed significantly modulated *Lactobacillus* spp., suggesting that active compounds in mangosteen pericarp and their derivatives may exert colonic microbial population effects, in agreement with Cardona et al. (2013). This might explain the improved laying performance observed in this study and suggests that MCE treatment would be a suitable method for raising laying quails in tropical countries.

It has been reported that providing mangosteen pericarp at a level of 1 g per kg diet to laying hens during a high production period significantly reduced blood triglyceride and yolk cholesterol concentrations. The lowering of yolk cholesterol level by mangosteen pericarp supplementation is consistent with the finding in this study that yolk cholesterol concentration linearly decreased from 15.22 to 12.84 mg per gram of yolk when MCE was given in drinking water. A lower concentration of yolk cholesterol in quails receiving MCE is associated with the ability of active compounds to regulate cholesterol biosynthesis. Following the observation of Zadernowski et al. (2009),

who showed that mangosteen is a source of xanthones and phenolic compounds such as α-mangostin and tannin. Chang et al. (2001) showed that secondary metabolites of mangosteen, especially tannins, could suppress the activity of hydroxy-methyl glutaryl CoA reductase (HMG-CoA reductase) in the mevalonate pathway. The decreasing HMG-Co A reductase will increase the catabolic metabolism of low-density lipoprotein and total cholesterol concentrations before being used for cholesterol biosynthesis in the liver, in agreement with our findings. Furthermore, the MCE at different dilutions had no harmful effect on the liver of the laying quails. Therefore, MCE could also be used to solve the problem of high cholesterol concentrations in egg yolks, representing a health benefit for consumers.

In conclusion, we found that mangosteen pericarp extract in the drinking water of laying quails raised in summer has positive effects on their laying performance and egg quality and regulates the balance of gut microbiota. Therefore, providing MCE in drinking water could be a potential approach in reducing the heat stress by strengthening the laying performance and immunity of high-producing laying quails without any detrimental effect on their metabolic profiles.

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CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

AUTHOR CONTRIBUTIONS

W. Boontiam designed the project, performed experiment and data analysis, drafted the manuscript. P. Kumari assisted with manuscript preparation. All authors discussed the results and comments on the manuscript.

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