*Original Article*

# Effect of heat stress and feed type on trimethylamine concentration and fishy odor of duck eggs

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*Abstract*

We investigated the effect of heat stress and feed type on the trimethylamine (TMA) concentration and the fishy odor of duck eggs (Study 1) and the relationship between the TMA level in serum and egg content (Study 2). In Study 1, pooled egg samples (n = 75) were divided by feed type (CP12, n = 24; CP18, n = 27; CP20, n = 24) and heat stress (no, n = 49; moderate, n = 15; severe, n = 11). The collected samples were obtained for TMA analysis and sensory testing. In Study 2, a total of 15 egg and 15 serum samples from no heat stress (n = 10) and moderate heat stress groups (n = 5) were included in the TMA analysis. In Study 1, the TMA concentration of egg yolk in the severe heat stress was higher than the moderate and no heat stress ($P$ = 0.034), while the CP20 was higher than the CP18 and CP12 ($P$ = 0.008). Heat stress and feed types did not affect the fishy odor rating score of egg yolk and egg white ($P$ > 0.05). The TMA concentration of egg yolk positively correlated with the fishy odor score ($P$ = 0.010). In study 2, the serum TMA concentration was positively correlated with egg yolk TMA ($P$ < 0.05). In conclusion, the heat stress and feed type affected the TMA concentration of egg yolk but not egg white. An increased egg TMA concentration was found in the ducks exposed to severe heat stress or consumed feed containing high choline. There was no effect of heat stress and feed types on the fishy odor of duck eggs. Lastly, the TMA concentration in egg yolk was positively correlated with the TMA concentration in the serum.

**Keywords:** Duck egg, Trimethylamine, Fishy odor, Heat stress, Feed type

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## Introduction

Duck eggs are an essential human nutrition source containing protein (12 – 16%) and essential lipids (35 – 37% of egg yolk) with low cholesterol levels (Lai *et al.*, 2010; Abeyrathne *et al.*, 2013). Among Asian countries, duck egg production is approximately 12 – 20% of total egg production (Ismoyowati and Sumarmono, 2019). However, duck egg consumption is still lower than that of chicken eggs due to the fishy odor characteristic of egg content. Trimethylamine (TMA) is the main substance associated with a fishy odor in duck egg content (Li *et al.*, 2019). In poultry, gut microorganisms can break down TMA precursors such as choline or polyunsaturated fatty acids, resulting in the production of TMA. This TMA is absorbed through the intestine and later transformed into an odorless compound by hepatic flavin monooxygenase 3 (FMO3) enzymes. (Yeung *et al.*, 2007; Wang *et al.*, 2012). Naturally, ducks have lower FMO3 activity than chickens, resulting in a higher level of TMA in duck egg yolk (Li *et al.*, 2019). However, choline also plays an important role in the synthesis of membrane phosphatidylcholine, which can prevent the fatty liver by decreasing excessive lipid accumulation (Cooke *et al.*, 2007; Pickens *et al.*, 2009). Dietary choline supplementation has the benefit of preventing perosis in young animals and enhancing the growth rate and the utilization of fat in the liver (Wen *et al.*, 2014). The supplementation of extra-high choline in a laying duck diet (4351 mg/kg) significantly increases the TMA level in duck egg yolk compared with a basal diet containing a lower concentration of choline (1390 mg/kg) (Li *et al.*, 2018). The Khaki Campbell crossbred duck is one of the duck breeds commonly used for egg production in Southeast Asia (Thongwittaya, 2007). In Khaki Campbell crossbred ducks, the recommended energy and protein levels in the laying period were at least 2700 kcal/kg and at least 16.5%, respectively (Thongwittaya and Tasaki, 1992). However, the recommended choline level for Khaki Campbell crossbreds has not been reported. In practice, the varied concentrations of choline in laying duck basal diets depend on their ingredients and have been found to be 800 mg/kg (Su *et al.*,2021), 1396 mg/kg (Li *et al.*, 2018), and 1430 mg/kg (Lien and Jan, 1999). Soybean meal and fish meal are the main protein sources of duck feed and contain a high level of phosphatidylcholine (Zeisel *et al.*, 2003). Thus, feed types containing different levels of nutrients, such as protein, in relation to the alteration of the choline level may influence the TMA level and fishy odor of the duck egg.

In nature, duck egg production and egg quality are influenced by the temperature and relative humidity of the environment (Ismoyowati *et al.*, 2020; Awad *et al.*, 2021). Heat stress from high temperature or high humidity induced changes in duck egg yolk quality, serum antioxidant parameters, and hepatic enzyme activities (Ma *et al.*, 2014; Chen *et al.*, 2021). Ducks exposed to heat stress also showed decreased weight and length of the oviduct and a decreased number of large follicles (Ma *et al.*, 2014). Thus, during the production period, the varied environmental conditions may influence the TMA level and the fishy odor of duck eggs. To date, studies on the effect of heat stress and feed type on TMA levels and the fishy odor of duck eggs have not been comprehensively investigated. Thus, the objective of the present study was to investigate the effect of heat stress and feed type on the trimethylamine (TMA) concentration in duck egg content and the relationship between the TMA level in the serum and the TMA level in the egg content.

## Materials and Methods

***Animal care and general farm management***: The experimental procedures were performed according to the Ethical Principles and Guidelines for the Use of Animals, National Research Council of Thailand, and approved by the School of Agricultural Resources, Chulalongkorn University animal care and use committee (protocol number IACUC001/2566). The studies were carried out on a farm located in the northern part of Thailand with 300 laying ducks during September, January, and April. The ducks were kept in conventional open-housing system pens consisting of a concrete floor indoor area and a ground floor outdoor area. Rice husks and straws were used as bedding. Only natural light was provided during the study. Study 1 aimed to investigate the effect of heat stress and feed type on the TMA concentration and fishy odor score of duck eggs. Study 2 aimed to evaluate the effect of heat stress on the TMA concentration in the serum in relation to the TMA concentration of duck eggs.

***Study 1***

***Experimental designs:*** A total of 180 Khaki Campbell crossbreds were included in the study. During the experiment, the ducks were 25 – 30 weeks old with 1.57 ± 0.21 kg of body weight. The ducks were divided by feed type containing different levels of crude protein (CP) (CP12, n = 60; CP18, n = 60, and CP20, n = 60). The animals were fed twice a day (170 g per animal per day). The nutritional composition of commercial feeds is presented in Table 1.

The ducks were nourished in pens (4 $m^2$ per animal) and allowed to freely access indoor and outdoor areas. A swimming channel was provided in the outdoor area. Drinking water was also provided *ad libitum* via a water bucket. The temperature and humidity of the environment were measured using a digital thermo-hygrometer. The devices were placed 30 cm above the floor at the center of the pen. The records included daily minimum and maximum, as well as current actual temperatures and humidity. The current actual temperature and humidity were recorded at 0600, 1200, 1800, and 2400 h. The actual temperature and relative humidity were used for temperature-humidity index (THI) calculation as per the following equation: THI = temperature (°C) - (0.31 - 0.31 x relative humidity (%)/100) x (temperature (°C) - 14.4) (Awad *et al.*,2020). The values of THI obtained from the calculation were classified as follows: no heat stress (THI < 27.8), moderate heat stress (27.8 ≤ THI < 28.9), and severe heat stress (THI ≥ 28.9) (Awad *et al.*,2020). The intensity of daylight in the pen was measured using a lux meter to measure the natural sunlight intensity 30 cm above the floor. The beginning and the ending of daylight were measured from 0500 – 0700 h and from 1700 – 1900 h, respectively. The descriptive statistics of the temperature-humidity index and duration of daylight during the experiment are presented in Table 2. The eggs were randomly collected daily to evaluate the TMA concentration and the fishy odor score.

***Sample collection:*** The total egg production per day was defined as the number of eggs collected at 0600 plus those collected at 1800 h. In each group of feed type, 5 eggs were randomly selected from all eggs produced per day for 1 − 2 weeks continuously. The egg weight, shell weight, and egg yolk weight of the selected eggs were measured using a digital weighing scale. The egg white weight was calculated as the egg weight minus the weight of the shell and egg yolk. Egg yolk (10 ml) from each selected egg was collected and pooled together. The pooled egg yolk samples (50 ml) were immediately stored at -20°C. Pooled egg white samples were collected using the same procedure as egg yolk.

***TMA analysis of the egg samples:*** The pooled egg yolk samples (n = 75) from each group of feed type were as follows: CP12 (n = 24), CP18 (n = 27), and CP20 (n = 24). The pooled egg white samples from each feed type were also obtained for the TMA analysis (n = 75). The TMA concentrations in the pooled samples were evaluated using the colorimetric method modified from a previous study (Li *et al.,* 2018). Briefly, the samples were precooled at 40°C for 24 h and placed at room temperature (25°C) for 4 h. The thawed samples (7.5 ml) were homogenized with 10 ml of 4°C precooled 10% (wt/vol) trichloroacetic acid (TCA) (Merck KGaA, Darmstadt, Germany) and left for 16 h at room temperature. The mixture was filtrated with Whatman #2 filter paper. The filtrated sample (2 ml) was mixed with 0.5 ml of 10% formaldehyde, 5 ml of toluene, and 1.5 ml of 0.5% (wt/vol) KOH and shaken for 2 h at 30°C. The toluene fraction (3.0 ml) was collected and gently mixed with 0.48 of anhydrous $N_2SO_4$ (QReC, Auckland, New Zealand) and left for 5 min at room temperature. The toluene fraction (1.5 ml) was mixed with 1.5 ml of 0.02% (wt/vol) picric acid. The TMA concentration in the mixture was measured with a spectrophotometer at 410 nm. A 10% TCA solution was used as a negative control. The 15 concentrations (1 – 20 ug/ml) of TMA in toluene (Tokyo Chemical Industry, Tokyo, Japan) were used to create a standard curve to calculate the TMA level.

***Fishy odor scoring test:*** The fishy odor scoring test was evaluated by 25 random untrained volunteers from the north (n = 10), the central (n = 10), the south (n = 2), and the northeastern regions (n = 3) of Thailand including 12 males and 13 females (21 – 30 years old) within two days. A total of 36 egg yolk and 36 egg white pooled samples were randomly selected. The samples were thawed at 4°C for 12 h at room temperature for 4 – 5 h before the sensory test. All volunteers evaluated each pooled sample. The samples were divided into 4 batches (9 samples per batch) and presented to the individual volunteer with 15 min rest between batches. Distilled water was used as a blank between the individual samples. Additionally, the fishy odor sensory test for the pooled egg white samples was assessed afterward, following the protocol used for egg yolk on the same day. The fishy odor intensity of the thawed egg yolk samples was evaluated and rated on a scale of 1 – 5 (i.e., 1 = absent, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong).

***Study 2***

***Experimental design and serum sample collection:*** The 27 ducks (30 weeks old and 1.67 ± 0.12 kg body weight) were included in the study. The ducks were individually kept in pens (3 $m^2$) with straw bedding located in the indoor area for 2 days (0 – 48 h). The animals were fed a commercial diet twice a day (170 g per animal per day). The commercial feed contained 316.3 kcal/100 g of diet and 18.0% crude protein. Water was provided *ad libitum* via a water bucket. Duck eggs were collected at 24 and 48 h. The ducks (n = 15) that produced eggs at 24 – 48 h of the experiment were randomly selected to perform blood collection of the individual animal at 48 h. The blood sample (3 – 4 ml) was collected once from the wing vein using a 20–gauge needle and was kept in the serum clot activator tubes. The blood sample was centrifuged at 1500 g for 10 min, and the collected serum was immediately stored at -20°C.

***Trimethylamine (TMA) analysis of egg and serum samples:*** The TMA concentration was evaluated in egg yolk and egg white using the colorimetric method, as described in Study 1. The TMA concentration in the duck serum samples was evaluated using the headspace gas chromatography (HS-GC) method, which was modified from a previous study (Wang *et al.,* 2012). The serum sample (1 ml) was added to the 50% (wt/vol) KOH (5 ml) in the 20 ml headspace vial and sealed immediately. The sealed vial was left at room temperature for 30 min, then the vial was placed in the static headspace autosampler 7697A (Agilent, CA, USA) at a 60°C oven temperature with a 15 min oven/vial equilibration time. Next, the headspace gas (1 ml) was injected into a 30 m × 0.25 mm × 0.25 um GC column HP-INNOWAX (Agilent, CA, USA). The analysis was performed using a gas chromatographer 7890B GC system (Agilent, CA, USA) equipped with a mass spectrometer 7000C GC/MS Triple Quad (Agilent, CA, USA). The injection was in split mode (split ratio 50:1) with a 200°C inlet temperature. The operating conditions were set with an initial oven condition of 40°C and 3 min holding time. After 3 min of holding, the temperature was ramped at a rate of 20°C /min rate to 180°C with a final hold time of 3 min. The data were obtained using the secondary ion monitoring (SIM) technique. The peak area from 0 – 12 min was recorded. For the identification of TMA, ion patterns 48, 58, and 59 were monitored. The identification was based on the ratio of ion 58:59 at approximately 1:0.5 and the retention time at approximately 1 – 2 min. The quantification of TMA was based on the response of ion 58. The TMA concentration in the sample was proximately quantified using the standard curve technique. The 4 standard TMA solutions diluted with 50% KOH (0.5 – 10 ug/ml) were used to obtain final concentrations of 0.5, 6.0, 9.0, and 10.0 ug/ml. A 50% KOH solution was used as a negative control.

**Table 1** Nutritional composition in different feed type groups

| Parameters | Feed type groups | | |
|---|---|---|---|
| | CP12 | CP18 | CP20 |
| Crude protein (g/100 g) | 12.6 | 18.2 | 20.0 |
| Energy (kcal/100 g) | 324.0 | 316.3 | 337.6 |
| Carbohydrate (%) | 57.3 | 52.7 | 55.6 |
| Total fat (g/100 g) | 5.0 | 3.6 | 6.1 |
| Fiber (g/100 g) | 22.6 | 6.2 | 4.5 |
| Choline (mg/kg) | 1320.3 | 1350.0 | 1414.6 |

**Table 2** Environmental characteristics in different heat stress groups (means ± SD)

| Parameters | Heat stress | | |
|---|---|---|---|
| | No | Moderate | Severe |
| **Temperature-humidity index** | | | |
| Average | 24.6 ± 2.9 | 28.2 ± 0.3 | 29.3 ± 0.3 |
| Range (min-max) | 14.5 − 27.7 | 27.8 − 28.8 | 28.9 − 29.9 |
| **Temperature (°C)** | | | |
| Average | 25.4 ± 3.0 | 29.4 ± 0.7 | 30.8 ± 0.6 |
| Range (min-max) | 14.7 − 29.7 | 28.2 − 30.8 | 29.7 − 30.7 |
| **Humidity (%)** | | | |
| Average | 81.7 ± 11.2 | 78.0 ± 10.9 | 72.7 ± 6.7 |
| Range (min-max) | 34.5 − 99.0 | 55.8 − 96.3 | 63.0 − 88.0 |
| **Duration of daylight (h)** | 12.5 ± 0.1 | 13.2 ± 0.2 | 13.5 ± 0.3 |

***Statistical analysis*:** Statistical analyses were carried out using SAS (SAS Inst. Inc., Cary, USA). Continuous data were analyzed using the MEANS procedure, and the data were presented as the mean ± standard deviation (SD). Univariate analysis was performed to evaluate the normality of the continuous variables. The normally distributed data were analyzed using parametric statistical procedures. The TMA concentration in the serum was normally distributed. The TMA concentration in the egg was distributed approximately normally. In Study 1, independent variables including heat stress (no heat stress, n = 49; moderate heat stress, n = 15 and severe heat stress, n = 11), feed type (CP12, n=24; CP18, n=27 and CP20, n=24) and their interaction were considered as main effects. The TMA concentration in the egg and fishy odor rating scores were considered dependent variables. In Study 2, heat stress was considered as the independent variable. The TMA concentration in the egg and serum were considered dependent variables. The correlation among the continuous parameters was evaluated using Pearson's correlation. In addition, multiple variance analyses were performed using GLM procedures to investigate the effect of the independent variables. Least-square means ± standard error of the mean (SEM) was obtained from each class of variables and was compared using the least significant difference test. $P < 0.05$ was considered statistically significant.

## *Result*

***Study 1***

***Descriptive statistics:*** On average, the egg production of the ducks was 76.23 ± 10.70% (range 67.7 – 94.1%). In the present study, egg production in the no heat stress, moderate heat stress, and severe heat stress groups were 79.46 ± 1.79%, 75.85 ± 3.27%, and 73.13 ± 4.63%, respectively.

Egg production in the CP20, CP18 and CP12 were 80.49 ± 1.62%, 75.83 ± 2.17% and 74.00 ± 3.37%, respectively. The characteristics of the selected eggs are presented in Table 3. The characteristics of the egg, including the egg weight, width, and length, were not significantly different within heat stress or feed type ($P > 0.05$). The egg yolk weight and egg white weight were not also different within heat stress groups ($P > 0.05$). However, the weight of the shell, egg yolk, and egg white were significantly different among groups of feed type ($P < 0.05$) (Table 3). In the no heat stress group, the shell weight was higher than the moderate and severe heat stress groups, respectively ($P < 0.01$).

**Table 3** Selected eggs characteristics by heat stress and feed type (Least-squares means ± SEM)

| Parameters | Heat stress | | | Feed type | | |
|---|---|---|---|---|---|---|
| | No | Moderate | Severe | CP12 | CP18 | CP20 |
| Weight (g) | 67.5 ± 0.3 | 66.2 ± 0.6 | 65.6 ± 0.7 | 66.6 ± 0.3 | 67.0 ± 0.3 | 67.3 ± 0.6 |
| Width (mm) | 44.3 ± 0.1 | 44.2 ± 0.3 | 44.6 ± 0.3 | 44.1 ± 0.3 | 44.2 ± 0.1 | 45.0 ± 0.4 |
| Length (mm) | 68.9 ± 8.1 | 61.2 ± 17.8 | 60.1 ± 18.3 | 60.8 ± 13.5 | 59.9 ± 10.4 | 59.6 ± 17.7 |
| Shell (g) | 8.3 ± 0.04$^{a}$ | 7.6 ± 0.09$^{b}$ | 7.9 ± 0.11$^{b}$ | 7.6 ± 0.08$^{x}$ | 8.4 ± 0.05$^{y}$ | 8.4 ± 0.09$^{y}$ |
| Yolk (g) | 22.3 ± 0.1 | 21.5 ± 0.3 | 21.6 ± 0.3 | 23.1 ± 0.2$^{x}$ | 21.7 ± 0.1$^{y}$ | 22.0 ± 0.2$^{y}$ |
| White (g) | 36.7 ± 0.2 | 36.9 ± 0.4 | 36.0 ± 0.5 | 35.9 ± 0.2$^{x}$ | 37.0 ± 0.3$^{y}$ | 37.4 ± 0.4$^{y}$ |

***Effect of heat stress and feed type on TMA concentration in the eggs:*** Overall, the TMA concentrations in the egg yolk and egg white were 7.55 ± 2.96 µg/g and 1.05 ± 0.96 µg/g, respectively. Heat stress and feed type affected the TMA concentration in egg yolk ($P < 0.05$) (Table 4). Among the heat stress groups, the TMA concentration of egg yolk in the severe heat stress was higher than in the moderate heat stress and no heat stress groups (9.96 ± 1.02 µg/g versus 7.90 ± 0.66 µg/g and 7.17 ± 0.34 µg/g, respectively, $P = 0.034$). The TMA concentration of egg yolk in the CP20 was higher than in the CP18 and CP12 groups (9.13 ± 0.58 µg/g versus 7.04 ± 0.41 µg/g and 6.99 ± 0.58 µg/g, respectively, $P = 0.008$). However, heat stress and feed type did not significantly affect the TMA concentration in egg whites ($P > 0.05$) (Table 4).

Moreover, the interaction between heat stress and feed type affected the TMA concentration in egg yolk ($P = 0.001$) (Fig. 1A). The TMA concentrations in egg yolk obtained from the severe heat stress group fed with 20% CP diet (12.39 ± 1.55 µg/g) was significantly higher than those obtained from the no heat stress group fed with 18% CP (6.41 ± 1.55 µg/g) and the moderate heat stress group fed with 12% CP diet (5.67 ± 0.19 µg/g) ($P < 0.05$). The interaction between heat stress and feed type also affected the TMA concentration in egg whites ($P = 0.002$) (Fig. 1B). There were only TMA concentrations in egg whites obtained from the no heat stress group fed with 20% CP feed (1.97 ± 0.22 µg/g) was significantly higher than the no heat stress group fed with 18% CP diet (0.74 ± 0.16 µg/g) ($P < 0.05$).

**Table 4** Effect of heat stress and feed type on trimethylamine (TMA) concentration in the eggs (Least-squares means ± SEM)

| Sample | Factors | *P* value | Classification | TMA (µg/g) |
|---|---|---|---|---|
| Egg yolk | Heat stress | < 0.05 | No | 7.17 ± 0.34 [a] |
| | | | Moderate | 7.90 ± 0.66 [a] |
| | | | Severe | 9.96 ± 1.02 [b] |
| | Feed type | < 0.01 | CP12 | 6.99 ± 0.58 [a] |
| | | | CP18 | 7.04 ± 0.41 [a] |
| | | | CP20 | 9.13 ± 0.58 [b] |
| Egg white | Heat stress | 0.061 | No | 1.09 ± 0.12 |
| | | | Moderate | 0.72 ± 0.27 |
| | | | Severe | 0.40 ± 0.38 |
| | Feed type | 0.093 | CP12 | 1.13 ± 0.21 |
| | | | CP18 | 1.00 ± 0.19 |
| | | | CP20 | 1.53 ± 0.21 |

[a, b] Different superscripts within a classified group of factors differ significantly ($P < 0.05$).

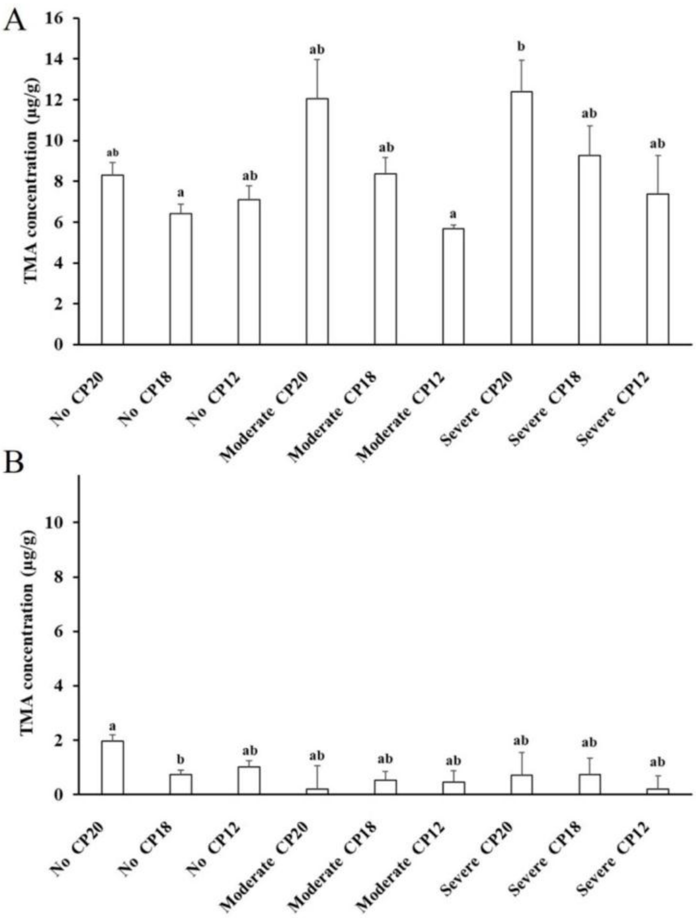


**Figure 1** The interaction effect between the heat stress and feed type on the trimethylamine (TMA) concentration in the egg yolk (A) and egg white (B). [a,b] Different superscripts among groups differ significantly ($P < 0.05$).

***Effect of heat stress and feed type on fishy odor rating score of the eggs:*** On average, the fishy odor rating scores for egg yolk and egg white were 2.37 ± 1.18 and 2.47 ± 1.37, respectively. In the present study, the heat stress did not significantly affect the fishy odor rating score of egg yolk and egg white ($P > 0.05$) (Table 5). However, the heat stress was most likely to affect the rating score of egg yolk significantly ($P = 0.058$) (Table 5). Moreover, the feed type also did not affect the rating score of egg yolk ($P = 0.524$) and egg white ($P = 0.921$).

In addition, there was no interaction effect between heat stress and feed type on either egg yolk ($P = 0.288$) or egg white ($P = 0.721$).

The relationship between TMA concentration and fishy odor rating scores of the egg yolk and egg white samples is presented in Table 6. The TMA concentration in the egg yolk was positively correlated with the average fishy odor rating score of the egg yolk (Score = 0.1442(TMA) + 1.0923, $P = 0.010$). However, the TMA concentration in egg yolk was not correlated with the fishy odor rating score of egg white ($P > 0.05$). Additionally, the TMA concentration in egg white was not correlated with the fishy odor rating score of egg yolk or egg white ($P > 0.05$).

***Study 2:*** On average, the serum TMA concentration was 1.47 ± 0.21 µg/ml (range 1.08 – 1.91 µg/ml). The serum TMA concentration was positively correlated with the egg yolk TMA concentration (Yolk TMA = 0.0749(Serum TMA) + 0.084, $P = 0.026$, Fig. 2). However, there was no correlation between the concentration of TMA in the serum and TMA in egg white ($P = 0.140$).

The egg characteristics and TMA concentrations in the egg samples by heat stress groups are presented in Table 7.

In study 2, the severe heat stress condition (THI ≥ 28.9) during the experiment was not detected. In the present study, the duck serum TMA concentration of no heat stress and moderate heat stress groups was not different (1.38 ± 0.10 and 1.51 ± 0.07, $P = 0.398$) (Fig. 3).

**Table 5** Effect of heat stress on fishy odor rating score of the eggs (Least-squares means ± SEM)

| Sample | Factors | *P* value | Classification | Rating score |
|---|---|---|---|---|
| Egg yolk | Heat stress | 0.058 | No | 2.28 ± 0.22 |
| | | | Moderate | 1.64 ± 0.56 |
| | | | Severe | 3.23 ± 0.46 |
| Egg white | Heat stress | 0.303 | No | 2.92 ± 0.28 |
| | | | Moderate | 3.50 ± 0.74 |
| | | | Severe | 2.00 ± 0.66 |

[a, b] Different superscripts within a classified group of factors differ significantly ($P < 0.05$).

**Table 6** Relationship between the trimethylamine (TMA) concentration and fishy odor rating score of the samples

| Parameters | TMA concentration | |
|---|---|---|
| | Egg yolk | Egg white |
| **Pearson's correlation coefficient (r)** | | |
| Fishy odor rating score of egg yolk | 0.347 * | 0.260 NS |
| Fishy odor rating score of egg white | 0.026 NS | 0.173 NS |

* Superscript correlated significantly ($P < 0.05$), NS = not significant

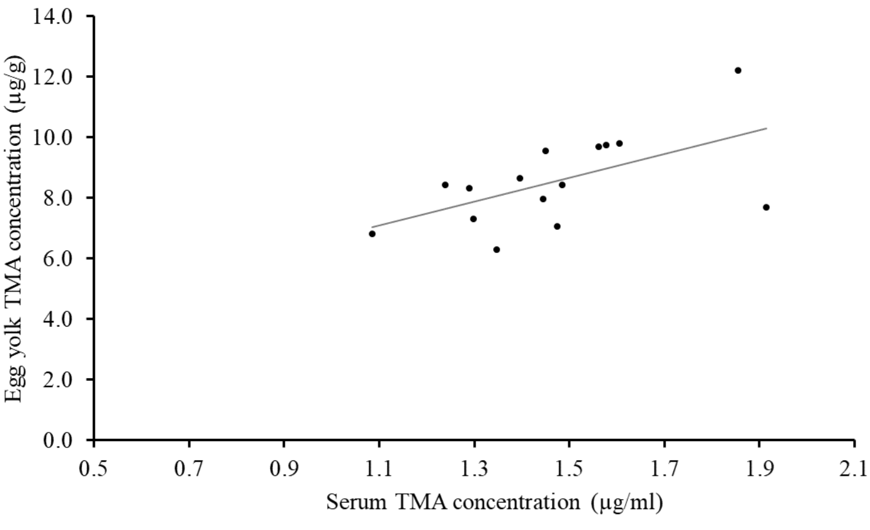


**Figure 2** Correlation between trimethylamine (TMA) in serum samples and egg yolk. Yolk TMA = 0.0749(serum TMA) + 0.084, ($R^2 = 0.3261$; $P = 0.026$).

**Table 7** Characteristics of selected eggs and trimethylamine (TMA) concentration in the egg from the ducks in different heat stress conditions.

| Parameters | No | Moderate | *P* value |
|---|---|---|---|
| Number | 10 | 5 | - |
| Egg weight (g) | 72.30 ± 1.29 | 69.93 ± 1.83 | 0.171 |
| Egg width (mm) | 45.52 ± 0.28 | 44.92 ± 0.40 | 0.901 |
| Length (mm) | 61.52 ± 0.75 | 61.06 ± 1.06 | 0.729 |
| Shell (g) | 8.85 ± 0.26 | 8.41 ± 0.37 | 0.358 |
| Egg yolk (g) | 23.41 ± 0.64 | 22.98 ± 0.90 | 0.158 |
| Egg white (g) | 39.01 ± 1.33 | 37.96 ± 1.88 | 0.626 |
| **TMA concentration** | | | |
| Egg yolk (µg/g) | 7.98 ± 0.67 | 8.70 ± 0.47 | 0.395 |
| Egg white (µg/g) | 1.05 ± 0.39 | 1.33 ± 0.28 | 0.572 |

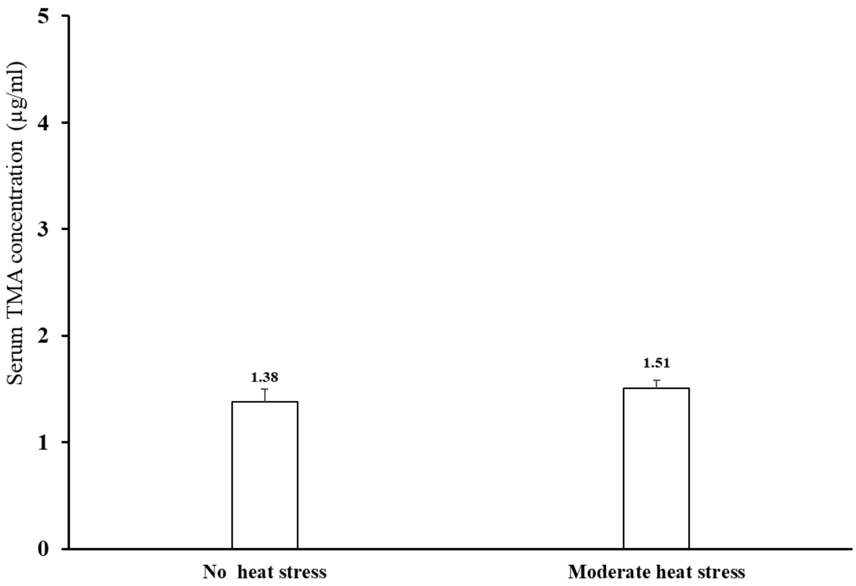


**Figure 3** Serum trimethylamine (TMA) concentration in different heat stress groups.

## *Discussion*

Overall, the present results reveal that heat stress and type of feed affect the TMA level in the duck egg yolk, which is positively correlated with TMA in the duck serum. In nature, the TMA level of egg white was quite low, and egg white was not the main deposition site of TMA in duck eggs (Pearodwong et al., 2022). Certainly, the present study did not find clear effects of independent factors, including heat stress or feed type, on TMA concentration and fishy odor of egg whites.

The changes in the gut microbial community or dysbiosis were found to have various consequences, including impaired digestibility in relation to the diminished growth rate, gastrointestinal disease, or change in metabolomics in the cecum (Zhang *et al.*, 2021; Zhang *et al.*, 2022). Heat stress impacts gut microbiota composition differences, mainly in the jejunum and cecum of ducks (He *et al.*, 2019). Heat stress also causes changes in intestinal morphologies, serum triglyceride, cholesterol, and total antioxidant levels and activities, which are associated with the dominance of TMA-producing bacteria (Firmicutes) in the jejunum (He *et al.*, 2019). A recent study clearly documented that the TMA-producing genus, *Ruminococcaceae_UCG_009*, was more abundant in the duodenal contents of ducks exposed to heat treatment (30 – 40 °C) compared to control ducks (Tian *et al.*, 2020). These findings corroborate the increase in TMA levels observed in the egg yolk of duck exposed to severe heat stress in the present study. In addition, the TMA metabolism pathway is quite complex and regulated by a combination of genetics and nutrition (Wang *et al.*, 2011; Bennett *et al.*, 2013). There have been reports on the impact of thermal conditioning in young poultry on the regulation of hepatic enzymes later in life (Ouchi *et al.*, 2021). Heat exposure induces changes in gene expression in the liver, leading to changes in the metabolic regulations of laying hens (Wang *et al.*, 2021) and laying ducks (Ma *et al.*, 2014). It suggests that the environment can impact alterations in FOM3 enzyme activities. Therefore, the effects of heat stress on FOM3 enzyme activity in ducks in relation to TMA level should be further investigated.

It has been reported that a hot climate negatively affects the blood profiles, including the serum lipid and antioxidant status of the duck (Awad *et al.*, 2021). Hot climate may subsequently influence the TMA levels in duck serum. In contrast, the current findings showed no significant difference in serum TMA levels between ducks that were exposed to heat stress conditions and those that were not. This could be attributed to the fact that the serum TMA concentrations obtained in the present study were derived exclusively from the groups subjected to no heat stress and moderate heat stress. The impact of heat stress conditions may become apparent if the serum obtained from the ducks exposed to severe heat stress is available for comparison.

The present result is consistent with a previous study that found a significant effect of diet on TMA levels in egg content, cecal content, and plasma. Laying hens fed with rapeseed meal have higher TMA levels in egg content and plasma than those fed with soybean meal (Wang *et al.*, 2016). Ducks that consumed a diet supplemented with fermented feed containing 18% crude protein exhibited lower TMA concentrations in their egg yolks compared to those on a basal diet containing 16% crude protein (Tian et al., 2024). This implies that protein level may not be the only factor affecting the TMA concentration among the different feed types. So far, the study on the effect of other nutrients on TMA levels in egg yolk has not been intensively investigated. Choline supplementation of 4000 mg/kg in the duck feed significantly increased 6.51 times of TMA level in duck egg yolk compared with control (Li *et al.*, 2018). In the present study, the choline level in the CP20 group was higher than in the CP18 and CP12 groups, around 60 – 100 mg/kg of feed. The average TMA level of egg yolk TMA obtained from the CP20 group was 1.2 – 1.3 times higher than the CP18 and CP12 groups, respectively. The higher choline level might be the comprehensive factor within the feed type affecting the TMA level in the egg yolk content.

Moreover, the level of TMA produced in the intestine also depends on the activities of TMA-producing bacteria (Li *et al.*, 2022). TMA-producing bacterial communities were also strongly influenced by diets (Rath *et al.*, 2020). Mammalians consuming different diets had different bacterial community compositions and relative abundances of gut bacteria encoding TMA-producing genes, namely choline-TMA lyase (CutC) and carnitine oxygenase (CntA) (Rath *et al.*, 2020). In ducks, the consumption of fermented feed had a significant impact on the composition of gut microbiota by reducing the relative abundance of *Ruminococcaceae_UCG_009* and *Ruminiclostridium_5* in comparison to the control (Tian et al., 2024). Therefore, in the current study, the impact of varying feed types on the changes in the relative abundance of intestinal bacteria producing TMA could be one of the factors influencing the TMA levels in egg yolks.

To date, there have been no reports regarding the influence of environmental conditions and feed type on the fishy odor rating score of duck eggs. However, in chickens, previous studies found a difference in the incidence of fishy odors in the eggs among seasons (Vondell, 1948; Hobson-Frohock *et al.*, 1973). Duck egg fishy odor perception can be affected by the gender and region of the evaluators (Li *et al.*, 2019). In the present study, the volunteers consisted of young adults (20–34 years old) from various regions of Thailand, and there was approximately equal balance between males and females. Nonetheless, the current results revealed no differences in the fishy odor rating scores of duck eggs among the groups exposed to heat stress and different feed types, despite the correlation between TMA concentration in the egg yolk and the fishy odor score. The range of egg yolk TMA levels observed in the previous study (1.0 – 33.0 µg/g) was wider than the range found in the current results (2.5 – 14.7 µg/g). (Li *et al.*, 2019). Duck eggs containing TMA, approximately 1 µg/g, mostly scored below 2 of 5 and scored a moderate to low fishy odor (Li *et al.*, 2019). In the present results, the range of mean yolk TMA among heat stress conditions and feed type ranged from 1.6 – 3.2 µg/g and 2.1 – 2.6 µg/g, respectively. Therefore, when exposed to low TMA concentrations or within a narrow range of TMA concentrations, humans may experience limitations in distinguishing fishy odors between samples.

It can be concluded that the heat stress and feed type affected the TMA concentration of egg yolk. There were no effects of heat stress or feed type on the TMA concentration of white eggs. An increased egg TMA concentration was found in the ducks exposed to severe heat stress (THI ≥ 28.9) or consumed feed containing high choline. However, neither heat stress nor feed type had an impact on the fishy odor of both egg yolk and egg white. TMA level in the serum obtained from no heat stress was not different from the moderate heat stress group. Lastly, the TMA concentration in the serum was positively correlated with the TMA concentration in egg yolk.

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***Conflict of interest:*** The authors declare that we have no conflicts of interest.

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