ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 15 (1): 8-14, 2016
ISSN 1682-8356

Effects of Red Mold Rice Produced from *Monascus purpureus* CMU002U on Growth Performances and Antioxidant Activity of Japanese Quail

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Abstract: This study researched the effects of red rice produced from San-Pah-Tong sticky rice (*Oryzae sativa* L.cv. Niew San-pah-tawng) and *Monascus purpureus* (Specie CMU002U). The study was divided into two parts. The first part consisted of a phytochemistry test and tests to determine ascorbic acid and phenolics. Terpenoids, triterpenoids, flavonoids, phenols, phlobatannins and coumarins were found. Phenolic and ascorbic acid levels were 3.00 µg ascorbic acid equivalent/g extract and 1.89 mg Gallic acid equivalent/g extract, respectively. The second part was an experiment using 75 Japanese quails in egg stage randomly divided into four groups as follows: the control group and groups in which each quail was fed with 6, 12 and 24 mg. red rice powder in capsules per day, respectively for 8 weeks. The Manlondialdehyde (MDA) from the serum and liver was then checked. It was found that the serum of the group which received 24 mg a day had reduced levels of MDA in the serum and liver. Compared with the control group, the difference was statistically significant at ($p<0.05$). After evaluation of body weight, consumption and survival rate of eggs in each group, no difference was found in body weight, but the control group consumed significantly more food than the others ($p<0.05$). Meanwhile, the group which received 12 mg a day produced significantly more eggs than the control group ($p<0.05$). Therefore, the antioxidants in red rice can reduce the amount of MDA in the serum and liver of Japanese quails. The fermentation of red rice can affect growth and production and it might be effective to decrease the toxicity of substances in Japanese quail.

Key words: Japanese quail, *Monascus purpureus*, antioxidants, red mold rice, poultry production

INTRODUCTION

Red rice is a product from rice with the fungus *Monascus* sp. which grew in the rice and released red pigment and many kinds of metabolites. One of the metabolites is Monacolin K, which has the effect of lowering blood lipid levels in people who suffer from high blood cholesterol (Lin, 2010) and animals which suffer from high blood cholesterol (Wang and Pan, 2003; Wang *et al*., 2006; Ho and Pan, 2009; Kumari *et al*., 2009; Rajasekaran and Kalaivani, 2011; Yeap *et al*., 2014; Bunnoy *et al*., 2015). Moreover, flavonoids can also be found, especially anthocyanin, which could eliminate free radicals in the superoxide and hydroxy 1 groups. This study aimed to study the antioxidant properties of San-Pah-Tong stingy rice (*Oryzae sativa* L.cv. Niew San-pah-tawng) and *Monascus purpureus* (Specie CMU002U). The laboratory for excellent academic and sustainable development of biological resources at the Faculty of Science, Chiang Mai University, produces high Monacolin K, also known as Mevinolin or Lovastatin, but in the fermentation process Hepato-nephrotoxic mycotoxin citrinin (CTN) also exists (Blanc *et al*., 1995). A study showed that the citrinin could cause toxicity in the liver and kidneys in mice, but it suggested that for levels which were not up to 200 ppm the function of the liver and kidneys would not be affected (Lee *et al*., 2010). Other studies showed that for Japanese quails in egg stage, it would not affect the growth and producing off eggs (Jirapinya *et al*., 2014) and it could reduce triglyceride in blood and meat in the chicken (Wang *et al*., 2006).

Free radicals are unstable compounds which are highly sensitive to chemical reactions with molecules around them, pulling electrons or molecules to make the radicals stable (Sies, 1991; Ames *et al*., 1993; Chattopadhyay and Chattopadhyay, 2008). In the body of an organism, free radicals are produced to face the stress, metabolism and the environment outside the body (Scott *et al*., 2005.). If more free radicals accumulated, oxidative stress is increased (Siu and Draper, 1982; Hagihara *et al*., 1984; Esterbauer *et al*., 1986; Birben *et al*., 2012) which mutates genetic material. The deterioration of tissue causes abnormalities in cells and tissues of living organisms (Bangchi and Puri, 1998). Meanwhile, the body uses enzyme antioxidants to control levels of free radicals. Therefore, the study of the antioxidant properties of red rice is the ultimate goal (Anggraini *et al*., 2015). Also, antioxidants have the potential to prevent liver damage and lipid peroxidation (Khennouf *et al*., 2010). Previous data has shown that red rice has antioxidants and a

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substance that reduces the amount of chlorate sterol, but the process affects the function of livers and kidneys. Therefore, if research verifies the effectiveness of red rice for producing antioxidant properties in the test tube and the quails, it might be possible to use it for raising animals, especially poultry that was at risk of free radical formation from the heat of the weather. As long as the red rice does not negatively affect the animals, this information could be useful for other research and red rice could be developed for raising animals.

MATERIALS AND METHODS

Red rice production in the laboratory: The red rice was made from the fermentation of San-Pah-Tong sticky rice (*Oryzae sativa* L.cv. Niew San-pah-tawng) and *Monascus purpureus* (CMU002U) for 14 days in the Laboratory for Excellent Academics and Sustainable Development of Biological Resources at the Faculty of Science, Chiang Mai University. To extract the red rice, 100 g of red rice mixed with 1,000 mg distilled water was soaked for 24 h, then sifted with a white cloth and filter paper No. 1. Next, the extract was evaporated until the mixture had a viscous texture in order to check for phytochemicals and antioxidants in a test tube. To produce red rice for feeding quails, after the fermentation the water was evaporated from the red rice at a temperature of 60 degrees for a night, was ground into a powder using a blender and packaged into capsules of 6, 12 and 24 mg.

Phytochemical testing: Terpenoids in the red rice and the extraction of red rice were measured by using the Salkowski test while alkaloids, sterols and triterpenoids were measured by using Mayer's test (Kasolo *et al*., 2010). Tannins, phenols, phlobatannins, anthraquinones, coumarins and flavonoids were measured by using a Ferric Chloride test (Trease and Evans, 2002).

Determination of antioxidant contents

Phenolic acid: Diluted Gallic acid (10-100 µg/ml) and the extract of red rice were absorbed into 1.5 ml of Folin Ciocalteu reagent with a 10 percent concentration of solution in the test tube, then 300 ml more of the extract or basic element gallic acid was added. Next, they were mixed and left in the dark for 3 minutes. After that, 1.2 ml of Na_2Co_3 was added and left in the dark for 30 min before being measured for absorbance using a spectrophotometer with 731 nm wavelength. Then, the results with gallic acid equivalent (GAE)/g extract were reported (Wolfe *et al*., 2003).

Ascorbic acid: Dinitrophenyl hydrazine (DNPH) reagent was prepared by mixing 2 g of dinitropheny hydrazine, 230 mg of thiourea, 270 mg of $CuSO_4.H_2O$ and 100 ml of 5 M H_2SO_4, stirring it together and storing it in a brown bottle at room temperature. After that, diluted ascorbic acid and red rice were absorbed into 150 µl of DNPH reagents mixed with 13.3% v/v TCA 200 µl in test tubes. Next, 600 ml of the extract or ascorbic acid was added and left at 37°C for 3 h and 1,000 ml of H_2SO_4 at 65% concentration was added into each test tube and mixed together. Finally, absorbance was measured using a spectrophotometer with 520 nm wavelength and the results were reported (Rani *et al*., 2004).

Quality testing of red rice in laboratory animals

Laboratory animals: Seventy-five Japanese quails (*Coturnix japonica*) in egg stage (35-day-old female) with 150 g weight in the TC quail farm were fed for 21 days before starting the experiment at the poultry division, Mae Jo University farm, Chiang Mai province. Feeding these laboratory animals was under the control of the Oversight Committee for the Animal Research Institute which utilized closed barns. Barns were kept at 60±5% relative humidity and a temperature of 26.5±2 degrees Celsius. The standard cage size was 50 x 52 x 42 cm with irrigation in every cage and animal food was used from Betagro Company which included not less than 22% protein, 13% humidity, 5% Fiber and 3% fat, while food and water were *ad libitum*.

Data collection: The food weight was recorded every day during the experiment and the body weight, amount of food, average egg production and survival rate were calculated by weighing each bird from each group. During the experiment, the egg-laying behavior and the amount of eggs were observed and recorded. After the end of the experiment, 5 ml. of blood was taken from each bird's wing vein to analyze and determine the amount of MDA in the serum and liver tissue.

Preparing liver tissue: Twenty mg of liver tissue was mixed with Phosphate buffer solution (PBS) 0.1M pH 7.4 and then a homogenized by centrifuging at 3,500 rpm for 10 min. The supernatants were collected and stored at 4°C for the next experiment (Shabbir *et al*., 2013).

Amount of MDA in serum and liver tissue by TBARS: The 0.1 ml. liquid from the serum and supernatants from the liver were prepared by adding 0.2 ml of Thiobarbituric acid (TBA) reagent, 0.45 ml of normal saline and 1.0 ml of Trichloroacetic acid (TCA) reagent and then boiling the mixture for 30 min. Then distilled water was added and the mixture was centrifuged at 3,500 rpm. The absorbance was measured with a UV-VIS spectrophotometer at 523 nm. Then the recorded data was calculated and compared (Buege and Aust, 1978). Tetramethoxypropane (TMP) was diluted with distilled water, 0.1 ml of diluted TMP was pipetted into the test tube and 0.45 ml of 85% normal saline and 0.1 ml of TCA reagent were added. After boiling for 30 min, 2.0 ml distilled water was added and the mixture was centrifuged at 3,500 rpm. After that, it was put into a

cuvette to measure absorbance with a UV-VIS spectrophotometer at 532 nm. The TMP standard graph was produced and used as the reference for determining the amount of MDA in the liver and serum (Buege and Aust, 1978).

Amount of protein by bradford: To prepare the bovine serum albumin (BSA) concentration at 10-100 µg/ml in 0.1 M PBS, BSA was put into the test tube, 1 ml of Bradford reagent was added and it was mixed. Then it was left for 5-10 min at room temperature. Absorbance was measured at 595 nm with a spectrophotometer. A standard graph of protein was performed. Then, the amount of protein in the serum and liver was read out by a standard graph of BSA (Bradford, 1976).

Experiment: This study utilized the Completely Randomized Design (CRD). The experiments were divided into four groups of three to five repeats. The first group was the control group which was fed with empty capsules. The second to the fourth group were fed with 6, 12 and 24 mg of red mold rice each quail per day, respectively for 8 weeks. The amount of feed intake, death and weight were continuously recorded during the experiment.

RESULTS

The results of the phytochemical test of the red rice and the extract of red rice showed terpenoids, triterpenoids, flavonoids, phenols, phlobatannins, coumarins and saponins. In terms of the amount of antioxidants, the phenolic and ascorbic acid content of the red rice extract was 1.89 mg GAE/g extract and 3.00 µg AAE/g extract, respectively (Table 1).

Amount of MDA in serum and liver tissue with TBARS: The amount of MDA, with TBARS assay, in the serum of the quails revealed that the control group had 4.13±1.27 mM/mg protein. However, the amount of MDA in the groups which were fed with 6, 12 and 24 mg of red rice a day were 4.27±0.42, 3.69±1.16 and 1.98±0.38 mM/mg, respectively (Fig. 1). Moreover, for the group receiving 24 mg/bird/day of red rice, the amount of MDA was significantly lower than the control group at ($p>0.05$). However, for the groups receiving 6 and 12 mg/bird/day, the amount of MDA was not significantly different from the control group at ($p>0.05$).

In addition, the amount of MDA with TBARS in the quails' livers in the control group and the groups which were fed with 6, 12 and 24 mg/bird/day of red rice contained 0.11±0.0151, 0.06±0.01, 0.061±0.01 and 0.06±0.03 mM/mg protein, respectively (Fig. 1). There was no significant difference in any group.

Amount of protein in serum and liver tissue of the quail fed with red rice: In terms of the amount of protein in the serum, it was found that the control group had 16.44±0.03 mg/µl serum. The other groups which were fed with 6, 12 and 24 mg of red rice had 15.98±0.09, 15.66±1.22 and 16.45±0.03 mg/µl serum, respectively (Fig. 2). There was no significant difference at ($p>0.05$). In terms of the amount of protein in the liver, it was found that the control group had 556.66±61.88 mg/mg while the other groups which were fed with 6, 12 and 24 mg of red rice had 6.10.66±13.50, 587.11±23.82 and 596.00±16.71, respectively (Fig. 2). There was no significant difference ($p>0.05$).

Table 1: Phytochemicals, antioxidants, phenolics and ascorbic acid (vitamin C) in a test tube of in red rice and the extract of red rice

Parameters	Red rice	Red mold rice extracted
Antioxident potentials		
Terpenoids	+	+
Alkaloids	-	-
Steroids	-	-
Triterpenoids	+	+
Tannins	-	-
Flavonoids	+	+
Phlobatannins	+	+
Coumarins	+	+
Anthraquinones	-	-
Saponin	+	+
Total phenolics content		
Phenolics (mg GAE-g extract)	ND	1.89±0.01
Vitamin C (µg AAE/g extract)	ND	3.00±0.05

Data are presented as Mean±SD; ND (not detected)

Table 2: Body weight of the variation of experimental Japanese quail

	Treatment groups			
	For feeding with RMR (mg/day/bird)			
Days	Control	6	12	24
0	191.00±14.40	184.07±14.80	177.93±19.20	180.40±16.40
7	188.07±14.50	184.60±12.30	185.80±15.70	188.27±10.60
14	190.13±19.80	193.73±16.30	190.47±19.10	186.27±11.60
21	191.13±16.90	195.67±13.60	188.60±14.70	199.07±16.50
28	188.27±15.40	191.80±13.50	186.60±14.60	194.53±16.30
35	184.47±16.40	187.80±12.90	181.67±12.70	191.07±17.00
42	181.33±18.20	183.20±12.30	176.80±14.80	186.20±18.60
48	175.93±10.10	182.53±13.20	175.27±17.50	177.47±14.10
52	180.58±12.90	184.51±13.60	177.91±18.00	184.91±14.60

Data are presented as mean ± SD. RMR: Red mold rice. a,b with differenct superscripts are significantly different ($p<0.05$)

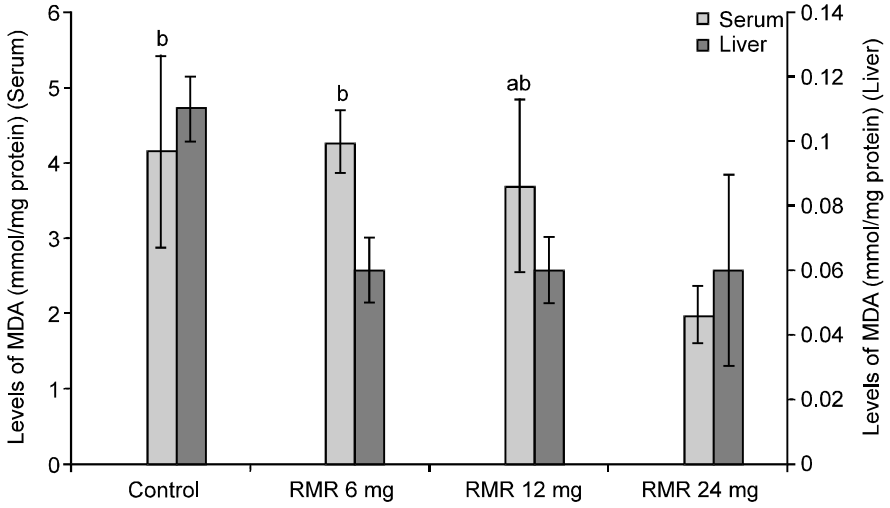

Fig. 1: MDA level in serum and liver with TBARS of variation of experimental Japanese quail. MDA: malondialdehyde, RMR: Red mold rice, Serum (thick) and Live (thin). a,b with differenct superscripts are significantly different ($p<0.05$)

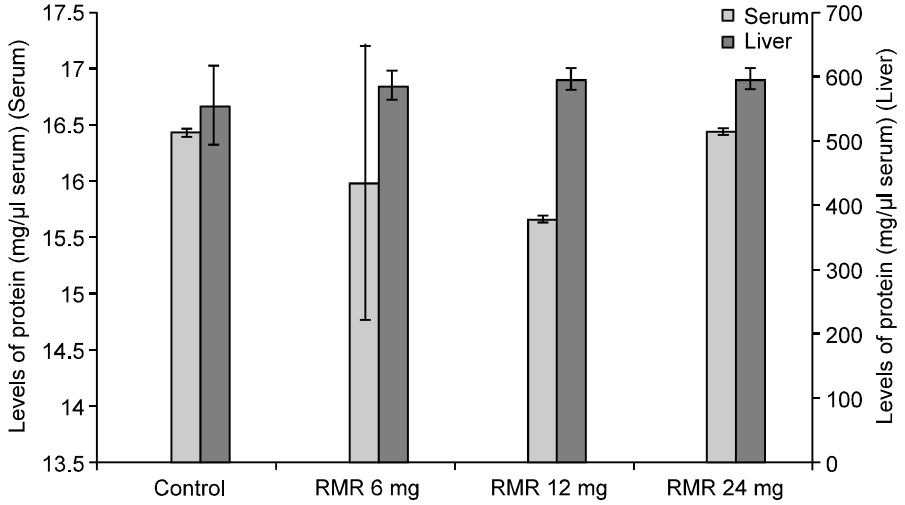

Fig. 2: Protein level in serum and liver with TBARS of variation of experimental Japanese quail. MDA: Malondialdehyde, RMR: Red mold rice, Serum (thick) and Live (thin)

Results of body weight, feed consumption, eggs production and death rate of Japanese quails: The quails were fed with red rice for 8 weeks and were weighed every week (Table 2). The results showed that the weights of the quails in each group from the beginning of the experiment to the end were similar to each other. They were not significantly different. In terms of the average body weight, the amount of food, eggs production and death rate (Table 3), it was found that the average body weights among the groups were not significantly different. Meanwhile, for the average feed consumption per day, the control group consumed 39.72 g/d while the other groups which were fed with 24 and 12 mg of red rice consumed 38.14 and 37.80 g/d respectively. Moreover, the group that was fed with 6 mg of red rice consumed the least at 35.73 g/d, which was significantly different at ($p>0.05$). In terms of egg production, all groups fed with red rice had a significantly higher rate than the control group ($p>0.05$). There was no death in any group.

DISCUSSION

Red rice has important phytochemicals which include terpenoids, triterpenoids, flavonoids, phenols, phlobatannins, coumarins and saponins. This finding is consistent with the study of Moko *et al.* (2014) in which the phytochemicals and antioxidants of red rice were found to have terpenoids, triterpenoids, flavonoids, phenols, phlobatannins, coumarins and saponins as the basic elements. Furthermore, from the testing of

Table 3: Body weight gain, average feed consumption, average egg production and mortality of the variation experimental Japanese quail

	Treatment groups			
		Fore feeding with RMR (mg/day/bird)		
Parameters	Control	6	12	24
Number of bird	15	15	15	15
Body weight gain	185.66±5.38	187.55±4.95	182.34±5.65	187.58±6.68
Average feed consumption (g/day)	39.73±0.30[a]	35.73±0.81[c]	37.80±1.14[b]	38.14±0.75[b]
Average egg production (%)	60.95.13±2.97[b]	75.83±1.35[a]	76.07±2.58[a]	75.00±1.89[a]
Mortality (%)	0	0	0	0

Data are presented as mean±SD. RMR: Red mold rice. a,b with differenct superscripts are significantly different ($p<0.05$)

antioxidants with 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, it was found that red rice could inhibit DPPH the most. This activity was also reported by Ham *et al.* (2013) which studied antioxidants and methanolic photochemistry in special rice species from Korea. There were phenols, anthocyanins, carotenoids, gamma-oryzanol and vitamin A. Moreover, red rice had the most antioxidants with DPPH radical scavenging activity. Phenolic acid is important for antioxidant activity because phenolic acid bonds with hydroxyl, peroxyl and superoxide directly in order to stop the chain reaction. Moreover, phenolic acid can increase the effectiveness of antioxidant enzymes and produce antioxidant proteins (Walter and Marchesan, 2011). It was also found that the level of phenolic acid in red rice was high at 1.89±0.01 mg GAE/g extract. When compared using the gallic acid equivalent, the phenolic acid levels of red rice in this study was higher than the standard for extract of red rice. Another important antioxidant is Vitamin C, which is the first compound that gives electrons to free radicals to make them become stable molecules and stop the chain reaction (Padayatty *et al*., 2003). The amount of vitamin C in red rice was 3.00±0.05 µg AAE/g extract when compared with ascorbic acid equivalent.

This can also be compared with the Veeru *et al.* (2009) study of antioxidant activity of the extract of plants used in medicine through extraction by methanol. These plants were *Desmodium ganeticum*, *Eclipta alba*, *Ocimum sanctum*, *Piper longum* (long paper), *Solanum nigrum* (European black nightshade) and *Amarunthus caudauts* (foxtail amaranth). Ascorbic acid was used to indicate the amount of antioxidants. It was found that all six plants had ascorbic acid between 3.86±0.20 to 21.33±1.49 mg/100 g. When compared with the phenolic acid and ascorbic acid content of red rice extract, it was found that red rice had fewer of those elements than the compared study. Red rice was found to be an antioxidant and could stop chain reactions (Walter and Marchesan, 2011). Furthermore, the study of Anggraini *et al.* (2015) found that red rice has high antioxidant activity and can prevent liver damage from lipid per-oxidation (Khennouf *et al*., 2010).

The results showed that red rice has antioxidant properties in the test tube. Then, the antioxidant effects of red rice in animals were tested and growth rate and egg production were considered. In terms of MDA testing, which was the biomarker, the red rice was good for lipid per-oxidation. It was found that for the group which was fed with 24 mg/d/bird of red rice, MDA in serum decreased, but the MDA in the liver did not differ significantly from the control group. Therefore, the results correspond to the study of Rajasekaran and Kalaivani (2011) which studied antioxidants and the decrease of fat in mice that had high cholesterol, which were fed red rice. The result of the DPPH radical scavenging assay was Ic50 was 250±0.2 g/ml. In addition, plasma lipid profiles and MDA, which indicated lipid per-oxidation, were not at normal levels. It was also found that 1.2 and 1.4 g/kg/day BW of the extract of red rice could reduce lipid per-oxidation by 33 and 31% respectively. Furthermore, there was reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. This could indicate antioxidants significantly higher than the control group. In terms of the effect on growth rate, red rice could be used for feeding quails because it did not increase the quails' body weight. This finding corresponds to the study of Jirapinya *et al.* (2014), who studied quails in egg stage. It was found that the body weights of the quails from the beginning to the end of the experiment remained similar to each other, but the amount of food consumed by group which was fed with red rice was different from the control group.

The animals which were used in this study were not negatively affected, as there were no deaths among the quails. This finding was related to the study of Wang *et al.* (2006), in which triglyceride could be reduced in chickens' blood and muscles. According to the above study, it suggested that citrinin, which results from fermentation, does not affect liver and kidney function of quails when the levels are less than 200 ppm. This is related to the study of Lee *et al.* (2010), which reported that Hepato-nephrotoxic citrinin which was less than 200 ppm did not the affect liver and kidney of mice during the experiment. That was because animals, especially birds, can control the balance of antioxidants. It also corresponded to the study of Khennouf *et al.* (2010), regarding the prevention of liver damage from lipid per-oxidation.

This study did not compare the oxidative stress of the control group and experimental groups. Therefore, a study about the oxidative stress is recommended, which could be done in the Laboratory for Excellent Academics and the Sustainable Development of Natural Resources, Faculty of Science, Chiang Mai University. It may provide evidence to support the idea that red rice has antioxidant properties which could reduce fat in quail and prevent oxidative stress.

Conclusion: Red rice has important phytochemicals including terpenoids, triterpenoids, flavonoids, phenols, phlobatannins, coumarins and saponins. Its phenolic and ascorbic acid contents were 1.89 mg GAE/g extract and 3.09 µg AAE/g extract, respectively. Red rice at quantities of twenty-four mg/d/bird had antioxidants that could reduce MDA in the quails' serum, but did not affect growth and egg production rate.

ACKNOWLEDGMENTS

This research was supported by Maejo University and Chiang Mai University. We wish to thank the Biotechnology Program, Faculty of Science, Maejo University and the Department of Biology, Faculty of Science, Chiang Mai University.

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