



# Vet Integr Sci

## Veterinary Integrative Sciences

ISSN: 2629-9968 (online)

Website: www.vet.cmu.ac.th/cmvj



### Research article

## Effect of dietary green tea extract supplementation in growing-finishing pigs on growth performance, meat quality, and oxidative stability of pork

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### Abstract

This experiment was conducted to investigate the effect of dietary supplementation with green tea extract on the growth performance, meat quality and oxidative stability of the *longissimus thoracis et lumborum* (LTL) muscle in growing-finishing pigs. A total of 16 crossbred pigs, half females and half males, were randomly assigned to 2 dietary treatments. This experiment lasted 9 weeks and included grower and finisher periods. The experimental diets in each period were a standard diet (control) and the standard diet supplemented with green tea extract at 500 mg/kg diet. Dietary supplementation with green tea extract did not affect the animal performance and meat quality parameters. However, dietary green tea extract improved the oxidative stability of the LTL muscle during storage at 4 °C for 6 d. Sensory characteristics were not impacted by green tea extract supplementation. In conclusion, dietary supplementation of pig diets with green tea extract at 500 mg/kg diet can be used to prevent lipid oxidation in pork.

**Keywords:** Green tea extract; Growing-finishing pigs; Growth performance; Meat quality; Oxidative stability

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**Article history;** received manuscript: 25 July 2022,  
revised manuscript: 8 August 2022,  
accepted manuscript: 16 August 2022,  
published online: 23 August 2022

**Academic editor;** Korakot Nganvongpanit



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## INTRODUCTION

The oxidation of meat lipids is a principal cause that deteriorates meat quality and reduces the shelf life of raw meat and meat products (Rather et al., 2016). It has a detrimental impact on the meat's nutritional value, flavor, and color (Rossi et al., 2013) and causes the possible production of toxic compounds such as aldehydes and peroxides (Augustin et al., 2008). Synthetic antioxidants have been employed to inhibit lipid oxidation in meat, but in the past few years, the need for natural antioxidants has arisen evidently because of the synthetic antioxidants' adverse side effects (Shah et al., 2014). For example, Goodman et al. (1990) indicated that butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are capable of exacerbating symptoms in some chronic urticaria patients. Many plant extracts, such as herbal extracts or essential oils, have demonstrated high antioxidant capacity and improved the oxidative stability of meat (Yesilbag et al., 2014; Hanczakowska et al., 2015; Cheng et al., 2017). Therefore, the majority of recent investigations have been focused on identifying natural antioxidants from different plant sources.

Green tea extract is a derivative of the cultivated evergreen tea plant (*Camellia sinensis* L.), which belongs to the Theaceae family (Perumalla and Hettiarachchy, 2011). It is a natural substance that has been demonstrated by *in vitro* studies to have substantial antioxidative (Rababah et al., 2004; Hu et al., 2009; Norkeaw et al., 2022), antimicrobial, and anti-inflammatory potential (Wang et al., 2011). Several polyphenolic compounds with antioxidative properties were found in green tea extract, but catechins (flavanols) are the most powerful active compounds found in green tea extract, with epicatechin-3-gallate and epigallocatechin-3-gallate being the most potent antioxidant compounds (Namal Senanayake, 2013). Many natural products derived from plants have been utilized in pig diets to promote growth performance and the immunological response (Yan et al., 2010; Li et al., 2012; Liu et al., 2013; Sampath et al., 2020), and improve the oxidative stability of pork (Rossi et al., 2013; Hanczakowska et al., 2015; Ranucci et al., 2015; Cheng et al., 2017). Dietary green tea extract supplementation (125 to 500 mg/kg diet) increased the oxidative stability of broiler chicken meat (Farahat et al., 2016). Nevertheless, few studies have investigated the effect of green tea extract supplementation in pig diets, and previous results regarding the improvement of the oxidative stability of pork are controversial. Hossain et al. (2012b) reported that supplementation of a finishing pig diet with green tea by-products (10 g/kg diet) enhanced the oxidative stability of pork. In contrast, Augustin et al. (2008) reported that supplementation of growing pig diets with green tea extract (32.4 or 323.6 mg/kg body weight/day) did not improve the oxidative stability of pork. The variation and bioavailability in plants is influenced by genetics (Zengin et al., 2018; Sobeh et al., 2019) and growing location (Yahyaoui et al., 2019). At present, investigating the effects of Thai green tea extract in pig diets is limited. Therefore, this study aimed to investigate the effects of Thai green tea extract supplementation in growing-finishing pig diets on the growth performance, meat quality, and oxidative stability of pork.

## MATERIALS AND METHODS

This research was carried out in conformity with the international and national guidelines for the care and use of research animals. The Animal Care and Use Committee at Chiang Mai University examined and approved all experimental protocols used in this work (2564/AG-0001).

### Green tea extract and raw materials

This study obtained green tea extract from Specialty Natural Product Co., Ltd., Thailand, while the other raw materials were purchased by Charoen Pokphand Group, Thailand.

### Animals, diets, and experimental design

Sixteen crossbred pigs ([Large White × Landrace] × Duroc) with an average live weight of  $51.5 \pm 3.6$  kg were used in this nine-week experiment. The pigs were randomly assigned on the basis of sex and weight to 2 dietary treatments, with 8 pigs per treatment (4 females and 4 castrated males). This experiment contained a 2-period feeding program, including grower and finisher periods, as shown in [Table 1](#). In each period, the pigs were fed 2 different experimental diets: a control diet and the control diet supplemented with green tea extract at 500 mg/kg diet (GTES). [Table 1](#) shows the ingredient and nutrient compositions of the diets. All experimental diets were formulated to meet or exceed the nutritional standards established by the National Research Council ([NRC, 2012](#)). This experiment was implemented in the Feed Research and Innovation Center of Charoen Pokphand Group, Chonburi, Thailand. The individual pigs were raised in identical concrete-floored pens with 2.0 m<sup>2</sup> of area. Each pen was equipped with a nipple drinker and a feeder that provided pigs ad libitum access to water and feed. Body weight and feed intake were individually measured and recorded at the beginning of the experiment and at the end of grower and finisher periods to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

**Table 1** The composition of experimental diets (as-fed basis).

Item	Grower (wk 0 to 4)		Finisher (wk 5 to 9)	
	Control	GTES	Control	GTES
Ingredient (%)				
Corn	14.19	14.14	25.77	25.72
Wheat	15.00	15.00	10.00	10.00
Barley	5.00	5.00	-	-
Cassava	20.00	20.00	20.00	20.00
Rice bran fine	15.00	15.00	15.00	15.00
Rice bran solvent	-	-	9.00	9.00
Soybean hull	-	-	5.00	5.00
Distillers dried grains	8.00	8.00	-	-
Canola meal	6.60	6.60	-	-
Soybean meal	6.30	6.30	10.10	10.10
Palm kernel meal	5.00	5.00	-	-
Animal protein	2.00	2.00	-	-
Molasses	1.00	1.00	2.00	2.00
Green tea extract	-	0.05	-	0.05
Limestone	0.70	0.70	1.21	1.21
Sodium chloride	0.31	0.31	0.38	0.38
Monocalcium phosphate	0.03	0.03	0.54	0.54
Vitamin and mineral premix <sup>1</sup>	1.00	1.00	1.00	1.00
Calculated nutrients				
Metabolizable energy (kcal/kg)	3,300	3,300	3,300	3,300
Crude protein (%)	15.75	15.75	12.72	12.72
Crude fat (%)	4.87	4.87	3.77	3.77
Crude fiber (%)	4.98	4.98	5.74	5.74
Calcium (%)	0.65	0.65	0.70	0.70
Total phosphorus (%)	0.67	0.67	0.70	0.70
Lysine (%)	1.00	1.00	0.90	0.90
Methionine (%)	0.30	0.30	0.28	0.28
Methionine + Cysteine (%)	0.58	0.58	0.50	0.50
Threonine (%)	0.62	0.62	0.56	0.56
Tryptophan (%)	0.18	0.18	0.15	0.15

Control = control diet; GTES = the control diet supplemented with green tea extract.

<sup>1</sup> Vitamin and mineral premix provided the following per kilogram of diet: 100 mg Fe; 90 mg Cu; 80 mg Zn; 60 mg Mn; 0.35 mg I; 0.25 mg Se; 6,000 IU vitamin A; 1,400 IU vitamin D3; 17 IU vitamin E; 35 mg niacin; 18 mg pantothenic acid; 2 mg vitamin B1; 8 mg vitamin B2; 0.05 mg vitamin B12; 0.15 mg biotin.

## Sample collection

At the end of the finisher period, all pigs were slaughtered in a commercial slaughterhouse according to the standard procedures. After fasting for 12 h, pigs were electrically stunned, exsanguinated, scalded, dehaired, eviscerated, and split in half. The carcasses were kept at 4 °C for 24 h, and then the LTL muscle samples were collected from the left side of the carcasses and stored at -20 °C for evaluation of meat quality and oxidative stability.

## Evaluation of meat quality

At 45 min and 24 h postmortem, pH values were determined in the LTL muscle between the 13<sup>th</sup> and 14<sup>th</sup> ribs (Jaturasitha et al., 2002) using a portable pH meter (Model 205, Testo, Lenzkirch, Germany). The pH meter was calibrated at ambient temperature by using a set of standard buffer solutions with known pH levels, including 4.01/7/9.21 (Mettler Toledo GmbH, Schwerzenbach, Switzerland). Measurement of meat color was conducted 48 h postmortem (Chaiwang et al., 2021) using a Konica Minolta Chroma Meter (model CR-400, Minolta Camera Co., Ltd., Osaka, Japan). The 2.5-cm-thick LTL muscles were oxygenated at room temperature for 30 min, and each muscle was then measured for the color on the surface area at three locations. Lightness (L\*), redness (a\*), and yellowness (b\*) were used to describe the color of meat. Before measuring the color of meat, the instrument was calibrated with a white tile that was unique to it and was programmed to use a D65 illuminant and a 2° observer with an 8 mm aperture (Barkley et al., 2018). The LTL muscles' chemical composition, consisting of moisture, crude protein, and crude fat, was determined in accordance with proximate analysis methods (AOAC, 2006). Drip loss was determined on 2.54-cm-thick slices cut from each LTL sample and used for exudate measurement. Each slice was weighed and sealed in a plastic bag. The samples were stored at 4 °C in the fridge for 24 h before the bags were opened and each slice was reweighed without the exudate (Tartrakoon et al., 2016). Furthermore, 200 g of each LTL sample was placed in a heat-resistant plastic bag and cooked in a water bath at 80 °C until the internal temperature of the sample reached 72 °C. The cooked samples were allowed to cool at room temperature and cut into 5 cubes (1 × 1 × 1 cm<sup>3</sup>) for evaluation of shear force (Chaiwang et al., 2021) using a texture analyzer (model TA.XT plus, Stable Microsystem, Ltd., London, England) with a Warner–Bratzler shearing device. The measurement was operated by shearing the cubes in a direction perpendicular to the core's long axis, and the peak force of the curve was considered as the shear force value (Choe et al., 2016).

A sensory evaluation of the grilled LTL slices was performed by a trained sensory panel consisting of eight people, following the method of Jaturasitha et al. (2002). Slices of the LTL sample (2.5 cm thick) without tendon and connective tissue were used. The slices were grilled in a convection oven (model 720, Mara, Taiwan) at 200 °C until the sample's internal temperature reached 70 °C, then cut into 8 small pieces (1.25 × 1.25 × 1.25 cm<sup>3</sup>) and served on pre-warmed plates. The panelists were requested to evaluate tenderness, juiciness, flavor, and overall acceptability by using a 9-point scale (9 = extremely tender, juicy, intense, and highly acceptable; 1 = extremely tough, dry, bland, and less acceptable).

## Evaluation of meat oxidative stability using the malondialdehyde (MDA) assay

After storing the LTL muscles at 4 °C in a fridge for 0, 3 and 6 d, the meat oxidative stability was evaluated using the MDA assay according to the method of Bergamo et al. (1998). A 2 g of meat was moved to a centrifuge tube and homogenized in a solution containing 4.75 mL of distilled water and 0.25 mL of 1,000 mg/L ethanolic BHT. Then, 0.5 mL of the homogenate was taken out and mixed with 0.5 mL of ice-cold 10% trichloroacetic acid (TCA). To eliminate proteins, the sample was aggressively mixed for 3 min and centrifuged at 10,000 g for 5 min. A 0.7 mL of the TBA solution, which contained 0.4% TBA in 2 mol/L acetate buffer at pH 3, was mixed with 0.3 mL of the supernatant. Following a degassing step, the mixture was heated for 30 minutes at 90 °C in a water bath. The sample was then cooled and the particulate material was eliminated by centrifuging it at 10,000 g for 5 min. Finally, 20 µL of sample was injected into the HPLC system. Analytical HPLC was performed following the technique of Sringarm et al. (2022) using an Agilent 1220 Infinity II liquid chromatography system equipped with an Agilent 1260 Infinity FLD spectra fluorescence detector (Agilent Technologies, Santa Clara, CA, USA). Reverse-phase column chromatography was performed using an Ultra Aqueous C18 column (250 × 4.6 mm, 5 mm) (RESTEK, Bellefonte, PA, USA). The mobile phase consisted of 2.5 mM sodium phosphate buffer (pH 7.0) and acetonitrile (50:50 v/v) at a flow rate of 1.0 mL/min. The fluorescence detector was set at  $\lambda$  Ex = 515 nm and  $\lambda$  Me = 543 nm. The total operating time was 5 min per sample. A standard curve made from various concentrations of MDA standard solution was used to calibrate the MDA levels in the samples. The results were expressed as mg MDA per kg meat.

## Statistical analysis

The statistical analysis of the data was performed using SPSS Statistics, v. 23.0 software (SPSS Inc., Chicago, IL, USA). The homogeneity of variance of all data was tested using one-way analysis of variance. The independent samples t-test was used to compare the means between treatments, and a level of  $P < 0.05$  was considered statistically significant. The results are displayed as the means and standard error of the mean (SEM).

## RESULTS

### Growth performance

All the animals remained healthy throughout the experiment. The growth performance parameters, including initial body weight, final body weight, ADG, ADFI, and FCR, are shown in Table 2. During the grower, finisher, and overall periods, there was no significant difference between the control and GTES groups in all of the growth performance parameters ( $P > 0.05$ ).

**Table 2** The growth performance of pigs fed control or green tea extract supplemented diets.

Item	Dietary treatments		SEM	P-value
	Control	GTES		
Grower (wk 0 to 4)				
Initial weight (kg)	51.75	51.25	0.899	0.792
Final weight (kg)	81.50	80.75	1.294	0.783
ADFI (g/d)	2,254	2,180	44.55	0.424
ADG (g/d)	992	983	21.50	0.847
FCR	2.286	2.226	0.056	0.612
Finisher (wk 5 to 9)				
Initial weight (kg)	81.50	80.75	1.294	0.783
Final weight (kg)	114.25	112.88	1.554	0.674
ADFI (g/d)	2,785	2,685	72.62	0.513
ADG (g/d)	936	918	18.41	0.646
FCR	2.973	2.936	0.062	0.774
Overall (wk 0 to 9)				
Initial weight (kg)	51.75	51.25	0.899	0.792
Final weight (kg)	114.25	112.88	1.554	0.674
ADFI (g/d)	2,540	2,452	57.67	0.465
ADG (g/d)	962	948	15.83	0.685
FCR	2.644	2.590	0.054	0.635

Control = control diet; GTES = the control diet supplemented with green tea extract; SEM = standard error of the mean; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio.

### Meat quality

The meat quality parameters consisting of pH values, meat color, chemical composition, drip loss, shear force, and sensory evaluation are presented in Table 3. Supplementation of green tea extract in growing-finishing pig diets did not affect any of the LTL muscle's meat quality parameters ( $P > 0.05$ ). The tendency of pH values at 24 h postmortem was lower than the pH values at 45 min postmortem in both treatments.



**Table 3** The meat quality of pigs fed control or green tea extract supplemented diets.

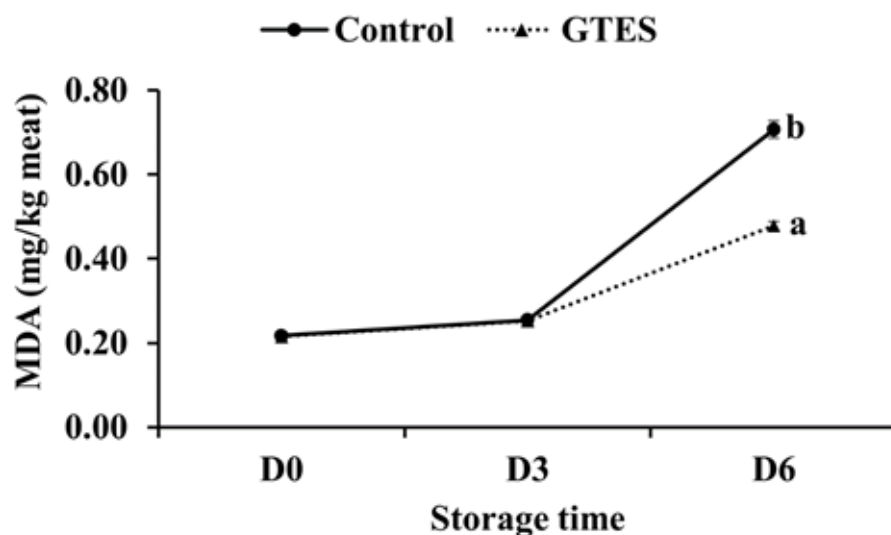
Item	Dietary treatments		SEM	P-value
	Control	GTES		
pH value				
pH, 45 min postmortem	6.42	6.38	0.039	0.632
pH, 24 h postmortem	5.73	5.62	0.033	0.084
Meat color				
Lightness (L*)	52.52	51.98	0.522	0.620
Redness (a*)	5.45	5.71	0.245	0.620
Yellowness (b*)	4.38	4.24	0.258	0.802
Chemical composition (%)				
Moisture	73.24	73.2	0.087	0.827
Crude protein	22.67	22.81	0.129	0.604
Crude fat	2.14	2.09	0.090	0.787
Drip loss (%)	3.87	4.02	0.188	0.693
Shear force (N/cm <sup>2</sup> )	38.08	37.17	1.259	0.732
Sensory evaluation				
Tenderness	6.11	6.41	0.122	0.226
Juiciness	5.63	5.83	0.138	0.462
Flavor	5.53	5.66	0.125	0.620
Overall acceptability	5.72	5.89	0.115	0.458

Control = control diet; GTES = the control diet supplemented with green tea extract; SEM = standard error of the mean.

### Meat oxidative stability

MDA, which is one of the ultimate products of lipid peroxidation, is normally used as an indicator of oxidative stability. A low MDA level indicates high oxidative stability. Figure 1 shows the effect of green tea extract supplementation in growing-finishing pig diets on the oxidative stability of the LTL muscle. There was no significant difference between the control and GTES groups in the meat MDA level at 0 and 3 d of storage time ( $P > 0.05$ ). However, at 6 d of storage time, the LTL muscle of pigs fed diets supplemented with green tea extracts had a lower MDA level than the control group ( $P < 0.01$ ).





**Figure 1** The meat oxidative stability of pigs fed control or green tea extract supplemented diets during refrigerated storage at 4 °C for 0, 3 and 6 d. MDA = malondialdehyde. Data are shown as the mean  $\pm$  standard error of the mean (SEM). Different letters in a time class demonstrate significant difference ( $P < 0.01$ ).

## DISCUSSION

Dietary green tea extract supplementation had no effect on the growth performance of growing-finishing pigs. The present findings agreed with that of [Augustin et al. \(2008\)](#), who reported that supplementation of green tea extract in a growing pig diet did not affect the growth performance. [Mason et al. \(2005\)](#) stated that the growth performance of finishing pigs was not impacted by dietary supplementation with green tea extract. In addition, supplementing the diet with 0.5% green tea by-product did not influence the final body weight, ADG, ADFI, and FCR of finishing pigs ([Hossain et al., 2012a](#); [Ko et al., 2008](#)). At the same time, [Yan et al. \(2021\)](#) indicated that supplementation of the Chinese local pig diet with tea powder had no effect on growth performance. Dietary supplementation of perilla cake, which its extract contains high levels of total phenolics and total flavonoids ([Chumphukam et al., 2018](#)), had no effect on the growth performance of finishing Thai crossbred black pigs ([Sringarm et al., 2022](#)). Several researchers have studied the use of plant extracts in growing-finishing pig diets, but no negative effects have been reported after their dietary supplementation on growth performance of those pigs ([Simitzis et al., 2010](#); [Rossi et al., 2013](#); [Hanczakowska et al., 2015](#); [Ranucci et al., 2015](#)).

The supplementation of growing-finishing pig diets with green tea extract had no impact on the chemical and physical parameters of meat quality. These findings are in agreement with that reported by [Augustin et al. \(2008\)](#), who indicated that dietary supplementation with green tea extract in growing pigs did not affect pH values, meat color, or drip loss in pork. [Mason et al. \(2005\)](#) indicated that supplementing green tea extract in weaning-finishing pig diets had no influence on meat color, moisture, crude protein, or intramuscular

fat. Furthermore, Hossain et al. (2012a) found that dietary supplementation with 0.5% green tea by-product in growing-finishing pigs did not influence cooking loss, chemical composition, shear force, or sensory evaluation of pork. Inclusion of perilla cake, which its extract showed potent antioxidant capability (Chumphukam et al., 2018), in a growing pig diet had no effect on meat quality (Arjin et al., 2021). In general, dietary supplementation with plant extracts has no influence on the physical or proximate quality of raw pork, as is the case for rosemary and garlic essential oils (Janz et al., 2007), grape seed extract (O'Grady et al., 2008), *Lippia* spp. leaf extract (Rossi et al., 2013), and a herbal extract mixture (Hanczakowska et al., 2015).

The flavor, color, and nutritional value of meat are all affected by lipid oxidation during storage (Rossi et al., 2013). Moreover, it reduces the meat's shelf life (Rather et al., 2016). The oxidative stability of meat is commonly investigated by measuring MDA, which is a major product of lipid peroxidation. In this study, dietary supplementation with green tea extract did not affect the meat MDA level after 0 and 3 d of storage. Nevertheless, after 6 d of storage, the LTL muscle of pigs fed diets supplemented with green tea extract had a lower MDA level than the LTL muscle of pigs fed control diets without green tea extract. In the studies of Ko et al. (2008) and Rossi et al. (2014), a substantial effect of pig diets on pork's lipid oxidation was observed after 3 d of storage at 4 °C. Green tea extract contains high total phenolic and total flavonoid levels (Norkeaw et al., 2022). These substances are regarded as possessing high antioxidant ability due to the redox characteristics of phenolic compounds, which are strong reducing agents via hydrogen donors and radical scavengers (Weerawatanakorn et al., 2018). Reports about the effect of dietary green tea extract supplementation on the oxidative stability of pig meat are limited. However, Hossain et al. (2012b), reported that supplementation of a finishing pig diet with green tea by-products significantly reduced the meat MDA level. A significant decrease in the meat MDA level was also found in broiler chickens fed green tea extract diets (Farahat et al., 2016), in contrast to the findings of Augustin et al. (2008), who observed that lipid oxidation in meat was not impacted by dietary supplementation of growing pigs with green tea extract (32.4 or 323.6 mg/kg body weight/day). Different inclusion levels of green tea extract in the diets and storage times may contribute to the varying findings. According to the results of this research, green tea extract can be added to pig diets to prevent lipid oxidation in pork.

## CONCLUSIONS

The findings of this study indicated that dietary supplementation of growing-finishing pigs with green tea extract inhibited lipid oxidation in pork without negative effects on pig performance and meat quality. Future studies are required to explore the capability of green tea extract supplementation in omega-3 polyunsaturated fatty acid-enriched pig diets, which are especially vulnerable to oxidization and reduce the oxidative stability of pork.

## ACKNOWLEDGEMENTS

The animals and feeds used in this research were supported by Charoen Pokphand Group, while the analytical laboratories were supported by Chiang Mai University, Chiang Mai, Thailand.

## AUTHOR CONTRIBUTIONS

Conceptualization, R.N. and K.S.; methodology, R.N., C.A., A.S., and P.H.; validation, N.C., M.T., and B.D.; data curation, R.N. and A.S.; formal analysis R.N. and T.Y.; investigation, R.N., C.A., and P.H.; project administration, K.S.; resources, S.M. and K.S.; writing-original draft, R.N.; writing-review and editing, R.N., C.A., S.M., and K.S. All authors read and agreed with the final manuscript.

## CONFLICT OF INTEREST

There are no conflicts of interest to disclose among the authors.

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#### How to cite this article;

Rakkiat Norkeaw, Chaiwat Arjin, Apinya Sartsook, Patipan Hnokaew, Marninphan Thongkham, Boonyarat Detruengsri, Niraporn Chaiwang, Supamit Mekchay, Terdsak Yano and Korawan Sringarm. Effect of dietary green tea extract supplementation in growing-finishing pigs on growth performance, meat quality, and oxidative stability of pork. *Veterinary Integrative Sciences.* 2022; 20(3): 571- 583.