JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0190071


≪Research Note≫

# Carcass Characteristics and Meat Quality of Betong Chicken Fed with Diets Supplemented with Crude Glycerin

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Experiments were conducted to evaluate the effect of crude glycerin inclusion in the diets of Betong chicken on the characteristics of their carcasses, internal organs, meat quality, lipid oxidation, and fatty acid profiles. One hundred 1-day-old chicks were raised for 8 weeks. Subsequently, the birds were sexed based on their morphological features, and weighed. Forty-eight male chickens, with comparable body weights, were randomly allotted to receive any of the three experimental diets, containing 0, 50 or 100 g crude glycerin/kg feed, on an as fed basis until they were 20 weeks old. A total of 24 chickens were slaughtered and their carcass characteristics and meat quality were studied. Results showed that carcass characteristics and internal organ parameters were not affected by crude glycerin supplementation ($P>0.05$). After chilling for 24 h, pH of the meat decreased in all groups ($P>0.05$), while shear force and cooking loss were not affected ($P>0.05$). Furthermore, crude glycerin did not affect the parameters such as crude protein, ether extract, ash, moisture and proportions of different fatty acid contents of meat of the Betong chicken ($P>0.05$). However, breast meat color and lipid oxidation were influenced by crude glycerin in diet ($P<0.05$). These results suggest that crude glycerin can be used at concentrations up to 10% in Betong chicken diet from 8 to 20 weeks of age. Nevertheless, its effect on breast meat color and lipid oxidation need to be considered.

**Key words**: Betong chicken, crude glycerin, meat quality

*J. Poult. Sci., 57: 291-296, 2020*

## Introduction

In Thailand, native chicken meat is popular among those consumers who prefers tasty and chewy meat. These chickens are also thought to have better meat quality, with higher protein content and less fat compared to imported broiler chicken (Wattanachant *et al*., 2004; Makchumpon *et al*., 2015). Betong chicken, a popular indigenous breed from southern Thailand, is well known for its higher growth rate and greater carcass weight as compared to other native chickens (Gongruttananun and Chotesangasa, 2001). Betong chickens take 16 to 20 weeks to reach the optimum weight of 1.6 to 2 kg (Chatreewong and Waree, 2006). Nevertheless, the cost of production of Betong and other native chickens is high, due to the high cost of feed. Therefore, an alternative feed source is one of the approaches to reduce production cost.

Crude glycerin (CG) is the principal by-product of biodiesel production, and it is used in pharmaceutical and cosmetic industry, livestock feed production, and food industries. However, purification for human need is very costly and not feasible for small and medium scale biodiesel producers (Thompson and He, 2006), particularly in Southeast Asian countries. Thus, the use of CG for livestock feed is probably the cheapest alternative. Several studies have confirmed that CG could substitute corn by 5 to 10% in the chicken feed without compromising growth performance and carcass yield in broiler chicken (Cerrate *et al*., 2006; Sehu *et al*., 2012; Topal and Ozdogan, 2013; Urgnani *et al*., 2014; Freitas *et al*., 2017; Da Silva *et al*., 2017; Garcia *et al*., 2018; Legawa *et al*., 2018) and meat quail (Arif *et al*., 2017; Farrapo *et al*., 2017). Although utilization of CG in broiler nutrition is widely reported, limited information is available regarding the effect of CG on meat quality, particularly in indigenous chicken breeds. Hence, the aim of the present study was to evaluate the effect of CG incorporation in the feed on carcass characteristics and meat quality of Betong chicken.

Received: June 3, 2019, Accepted: January 12, 2020
Released Online Advance Publication: March 25, 2020
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## Materials and Methods

***Birds, Housing and Feeding Treatments***

A total of 100 mixed-sex 1-day-old Betong chicks with an average body weight of 28.01±3.3 g were obtained from poultry hatchery, Department of Animal Science, Prince of Songkla University. These were raised under the same condition as in the hatchery and were fed a starter diet (19% CP and 3200 kcal/kg ME) formulated based on Nguyen and Bunchasak (2005) recommendations, over 8 weeks inside an open-sided naturally ventilated housing. Subsequently, the birds were sexed based on their morphological features and weighed. Forty-eight selected male chickens with similar body weight (708±84) were randomly assigned to 3 feeding treatments (0, 50 or 100 g CG/kg; designated as CG0, CG5, and CG10, respectively) and put in metabolic cages (46×21 ×36 $cm^3$ width, length, and height, respectively), using 16 birds with one bird per replication. These roosters were housed under 16 h artificial light and provided *ad libitum* access to water and their designated diet. The ambient temperature ranged from 26.5 to 31℃ and relative humidity was between 67.5 and 80%. They were vaccinated against Newcastle disease and infectious bronchitis at 1, 6 and 14 weeks, infectious bursal disease at 2 weeks of age and fowlpox when they were 3 weeks old.

The CG originating from waste vegetable oil was obtained from the Specialized Research and Development Center for Alternative Energy from Palm Oil and Oil Crops, Prince of Songkla University. The chemical composition of CG was previously evaluated by Sopian *et al*. (2018). The feed was based on a corn-soybean meal with 19% CP and 3,000 kcal/kg ME (in mash form) formulated according to Nguyen and Bunchasak (2010) recommendation. All experimental procedures were approved by Institutional Animal Care and Use Committee, Prince of Songkla University (MOE 0521.11/484). The feed composition and fatty acid profile are shown in Table 1 and Table S1 (Supplemental data), respectively.

***Carcass Characteristics and Meat Quality***

At the end of the rearing period (20 week), from each treatment group, eight chickens with body weight closest to the median, were starved overnight, weighed, and slaughtered according to the Islamic procedure of Thai Agricultural Standard 8400-2007 (ACFS, 2007). Subsequently, carcass and internal organs were weighed, and chilled carcass and slaughter weights were compared. The pH values and meat color were the mean of the three measurements, at the right breast muscle. The pH was measured using a pH meter (Seven2Go, Mettler-Toledo, Switzerland) at 45 min (pH45 min) and 24 h (pH24 h) post-slaughter. The color was evaluated using a colorimeter (Konica Minolta, Japan) and reported in the complete International Commission on Illumination (CIE) system color for the lightness (L*), redness (a*), and yellowness (b*). The breast meat samples were immediately transferred to and stored at −20℃ for chemical and fatty acid analyses.

Table 1. **Composition of experimental diets and their nutrient contents (as fed)**

| Items | Treatments[2] | | |
|---|---|---|---|
| | CG0 | CG5 | CG10 |
| Ingredients, % | | | |
| Corn | 58.17 | 52.61 | 47.77 |
| Palm oil | 1.00 | 1.00 | 1.00 |
| Soybean meal (42% CP) | 20.82 | 21.38 | 21.22 |
| Fish meal (55% CP) | 8.50 | 8.50 | 8.50 |
| Dicalcium phosphate | 1.00 | 1.00 | 1.00 |
| DL-methionine | 0.01 | 0.01 | 0.01 |
| Broiler Premix[1] | 0.50 | 0.50 | 0.50 |
| Rice bran | 10.00 | 10.00 | 10.00 |
| CG | 0.00 | 5.00 | 10.00 |
| Calculated content, % unless stated otherwise | | | |
| Metabolizable energy, kcal/kg | 3,000 | 3,000 | 3,000 |
| Crude protein | 19 | 19 | 19 |
| Ether extract | 6.6 | 7.18 | 7.74 |
| Dry matter | 88.51 | 88.31 | 88.11 |
| Ash | 5.69 | 5.96 | 6.18 |
| Lysine | 1.05 | 1.06 | 1.04 |
| Methionine | 0.39 | 0.38 | 0.38 |
| Calcium | 0.97 | 0.97 | 0.97 |
| Available phosphorus | 0.45 | 0.44 | 0.44 |

[1] Mineral-vitamin premix given values are per kg diet: Vitamin A 2,000,000 IU, vitamin $B_1$ 220 mg, vitamin $B_2$ 450 mg, vitamin $B_{12}$ 4.5 mg, vitamin $D_3$ 320,000 IU, vitamin E 2,000 mg, vitamin $K_3$ 330 mg, nicotinic acid 600 mg, Fe 10,000 mg, Cu 100 mg, iodine 150 mg, Zn 8,800 mg, Mn 8,800 mg, Co 130 mg.

[2] Abbreviations: CG, crude glycerin; CG0, 0% CG in diet; CG5, 5% CG in diet; CG10, 10% CG in diet.

To determine cooking loss, the left part of each breast muscle sample was cut into small pieces (2.0×1.0×0.5 cm), put in a sealed plastic bag and cooked in a water bath at 80℃ for 10 min, cooled and weighed. The weight before and after cooking were compared for each sample and presented as percent cooking loss. The same meat samples were subjected to shear-force analysis by Texture Analyzer (TA-XT2i, Texture expert Vision 1.17, Stable Micro System, Godalming, Surrey, UK). The operating parameters consisted of a cross-head speed of 2 mm/s and a 50-kg load cell (Wattanachant *et al*., 2004). The proximate analysis (dry matter, crude protein (CP), ether extract (EE), and ash) was performed according to AOAC (2006). The measurements for each sample were performed in duplicate.

Thiobarbituric acid-reactive substances (TBARS), a byproduct of lipid peroxidation, were estimated following the method described by Buege and Aust (1978). Briefly, 1.5 g of ground meat samples were mixed with 7.5 m*l* of TBA reagent (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N hydrochloric acid) and homogenized at 3000 rpm for 2 min (IKA-T18 Ultra Turrax, IKA®-werke GmbH & Co. KG, Staufen, Germany). Subsequently, the samples were heated in a boiling water bath for 10 min and cooled under running tap water. These were then centrifuged for 20 min at 3600×g (Hettich-Universal-320R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The absorbance of supernatants were measured at 532 nm using UV-spectrophotometer (Evolution 260 Bio, Thermo Fisher Scientific, Waltham, MA, USA) against blank. The results were compared to a standard curve of absorbance of 1,1,3,3-tetramethoxypropane and expressed as mg malonaldehyde (MDA) per kg of meat.

Total lipids were extracted with a mixture of chloroform and methanol (2:1, v/v) according to the method of Folch *et al*. (1957) and methylated as described by Metcalfe *et al*. (1966). The fatty acid methyl esters were quantified by gas chromatography (Hewlett-Packard 7890A; Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and a Varian SP-2560 capillary column (100 m length×0.25 mm internal diameter×0.2 $\mu$m film thickness). The initial column temperature was 70℃ held for 4 min, increased to 175℃ (13 C/min) held for 27 min, increased to 215℃ (4 C/min) held for 17 min and increased to 240℃ (4℃/min) held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The temperatures of the injector and detector were 240℃ and 250℃, respectively.

***Data Analysis***

Individual birds were used as an experimental unit and the data were analyzed using one-way ANOVA with SPSS version 16 (SPSS, 2007). The significance of differences were analyzed further with Tukey test. The statistical significance level was set at $P<0.05$ for all analyses.

Table 2. **Live weight, relative organ weight, and carcass composition of Betong chicken fed with different amounts of crude glycerin**

| Items | Treatments[1] | | | SEM | P-value |
|---|---|---|---|---|---|
| | CG0 | CG5 | CG10 | | |
| Live weight[2], g | | | | | |
| Initial weight (8-wks-old) | 708 | 706 | 708 | 12.18 | 0.998 |
| Final weight (20-wks-old) | 2195 | 2251 | 2277 | 29.61 | 0.534 |
| Slaughter weight[3], g | 2107 | 2175 | 2212 | 30.98 | 0.393 |
| Chilled carcass weight, g | 1810 | 1849 | 1872 | 25.68 | 0.631 |
| Relative organ weight[4], % | | | | | |
| *Pectoralis major* | 17.91 | 18.29 | 17.97 | 0.31 | 0.880 |
| Drumsticks with thighs | 32.97 | 32.57 | 31.96 | 0.41 | 0.628 |
| Wings | 9.29 | 9.09 | 9.44 | 0.07 | 0.095 |
| *Pectoralis minor* | 4.07 | 4.30 | 4.24 | 0.10 | 0.683 |
| Carcass composition[4], % | | | | | |
| Meat | 43.55 | 44.95 | 43.00 | 0.66 | 0.498 |
| Bone | 40.09 | 40.08 | 41.18 | 0.43 | 0.522 |
| Skin | 11.00 | 11.87 | 11.90 | 0.21 | 0.132 |
| Fat | 2.32 | 2.27 | 2.72 | 0.37 | 0.879 |
| Internal organ weight[5] | | | | | |
| Liver | 1.16 | 1.19 | 1.22 | 0.03 | 0.780 |
| Heart | 0.55 | 0.54 | 0.58 | 0.01 | 0.400 |
| Gizzard | 1.43 | 1.51 | 1.48 | 0.05 | 0.781 |
| Abdominal Fat | 0.84 | 0.72 | 1.38 | 0.23 | 0.299 |

[1] Abbreviations: CG, crude glycerin; CG0, 0% CG in diet; CG5, 5% CG in diet; CG10, 10% CG in diet; SEM, pooled standard error of the means.
[2] Each value represents the mean of 16 replicate per treatment
[3] Each value represents the mean of 8 replicate per treatment
[4] Values based on chilled carcass weight
[5] Values based on slaughter weight

Table 3. **Quality of breast meat from Betong chicken fed with diets containing different amounts of crude glycerin**

| Items | Treatments[1] | | | SEM | P-value |
|---|---|---|---|---|---|
| | CG0 | CG5 | CG10 | | |
| pH45 min | 6.27[a] | 6.11[b] | 6.29[a] | 0.03 | 0.001 |
| pH24 h | 5.97 | 6.01 | 6.00 | 0.02 | 0.747 |
| Breast muscle color | | | | | |
| L* (lightness) | 49.71 | 48.50 | 48.40 | 0.28 | 0.142 |
| a*(redness) | 1.62[a] | 1.38[ab] | 0.84[b] | 0.13 | 0.046 |
| b*(yellowness) | 8.20[a] | 6.25[b] | 6.11[b] | 0.29 | 0.003 |
| Shear force, kgf/cm$^2$ | 4.31 | 4.52 | 4.46 | 0.25 | 0.944 |
| Cooking loss, % | 14.17 | 15.00 | 15.83 | 0.40 | 0.246 |

[a, b] Values within a row with different superscripts are significantly different ($P<0.05$)
[1] Abbreviations: CG, crude glycerin; CG0, 0% CG in diet; CG5, 5% CG in diet; CG10, 10% CG in diet; SEM, pooled standard error of the means

## Results

The effect of treatments on live weight, chilled carcass, relative organ weight (breast, drumsticks with thighs, wings, fillet, liver, heart, gizzard, and abdominal fat), and carcass composition (meat, bone, skin, and fat) is shown in Table 2. CG supplementation had no effect ($P>0.05$) on all these parameters.

As shown in Table 3, the lowest pH45 min (pH 6.11) was observed ($P<0.05$) in samples from birds fed with 5% CG. After 24 h chilling, pH decreased in samples from all treatments and showed no significant difference ($P>0.05$) among the three groups. Shear force and cooking loss were not affected ($P>0.05$) by CG in the diet. However, breast meat color in term of yellowness, and redness were influenced by CG inclusion ($P<0.05$) but not the lightness ($P>0.05$).

The highest MDA values, a marker of lipid oxidation, of meat samples were found in CG10 group followed by CG5 and CG0 groups ($P<0.01$). The EE, which is the measure of total crude fat, from breast meat of the birds fed with CG had numerically higher values (CG5: 2.01%, CG10: 2.23%) than those fed with control diet (CG0: 1.91%), but these were not statistically significant. The results also showed that CG did not affect CP, ash and moisture contents of the meat ($P>0.05$). Moreover, the fatty acid composition of the breast meat was not altered by CG except for the stearic acid content, which reduced significantly.

## Discussion

The carcass characteristics and parameters of internal organs were not affected by CG supplementation. This could be attributed to the energy content in the CG and proved that CG was suitable as energy source for Betong chicken breed. The CG used in the current study, was previously evaluated to minimize the energy overestimation (Sopian *et al*., 2018). Therefore, these results were expected, as the feed was formulated to be isonitrogenous and isocaloric diet. These findings are in agreement with the previous studies which have reported that CG inclusion up to 10% had no negative effect on carcass traits (Sehu *et al*., 2012; Freitas *et al*., 2017) and on internal organs (Topal and Ozdogan, 2013). Similarly, Arif *et al*. (2017) and Farrapo *et al*. (2017) also found that CG did not alter the carcass characteristics and relative organ weight of quail. In contrast, Cerrate *et al*. (2006) reported several adverse effects on carcass yield and internal organs when 10% CG was used. Legawa *et al*. (2018) found increased abdominal fat in broilers fed with 5% CG. However, the former authors stressed that reduced carcass yield was related to a feed flow problem that led to reduced feed intake; contrastingly, the latter authors explained that the high abdominal fat content was due to a high content of fat in their CG. Therefore, it is important to evaluate the nutrient content of CG before it is mixed into the feed.

In the present study, the meat quality was not affected by experimental diets, except the pH45 min and meat color. However, pH24 h was not significantly different among samples from different groups. These values were similar to those reported by Bungsrisawat *et al*. (2018) in Betong chicken (KU line). Shear force and cooking loss were also not affected by CG. These results were consistent with the study using broiler chickens (Urgnani *et al*., 2014; Garcia *et al*., 2018; Legawa *et al*., 2018). However, in the present study, shear force values were greater and cooking loss values were lesser than those reported for 16-week-old Thai indigenous chicken (Wattanachant *et al*., 2004), and these might be related to the differences in the strain and the age of chickens used in these two studies.

The lower values of yellowness and redness of the meat were observed in CG-fed birds. The reduced meat color may simply be due to the lower corn content in these formulations (5.56% and 10.4% in CG5 and CG10, respectively), as compared to the basal diet. Peng *et al*. (2017) have reported that poultry diets have a great impact on the color of meat. It is well known that corn contains xanthophylls and other carotenoids that are the primary sources of pigments. This result was consistent with previous findings (Garcia *et al*., 2018) that in broiler chicken thigh meat yellowness value decreased linearly, with increasing levels of mixed semi-purified glycerin in diet. However, the same authors also found that breast meat color was not affected by the dietary glycerin content. Similarly, Urgnani *et al*. (2014) and Legawa *et al*. (2018) also did not find any significant change in the color of the breast muscles of broilers due to CG in their feed. These observations suggest that inconsistencies may arise because of differences in the genotypes of the birds, as well as difference in the source and levels of CG used in these studies.

To evaluate the oxidative stability of the meat samples, amount of TBARS was measured. The results suggested that compared to basic diet, inclusion of CG in the feed promoted significantly higher levels of MDA in the breast meat, which has higher fat and unsaturated fatty acid contents. Present results support Kovalík *et al*. (2018), who suggested that the susceptibility of meat to lipid oxidation increases with increasing unsaturated fatty acid contents, and Legawa *et al*. (2018), who concluded that level of MDA is directly dependent on the amount of fat in the meat. However, the oxidative stability of the meat can be enhanced by including antioxidants in the feed (Marzoni *et al*., 2014). No significant differences in the other chemical composition were observed among different experimental groups in the present study. The study by Silva *et al*. (2017) also confirmed that CG in diet did not affect EE, CP, ash and moisture content of the breast meat. Conversely, there are other studies which showed a higher EE content in breast meat (Legawa *et al*., 2018) and lower EE in thigh meat (Topal and Ozdogan, 2013) of broiler chicken, fed with CG. Authors of these reports proposed that these results were related to the EE content of the diets. However, the difference in the levels of EE in the different experimental diets in this study was insufficient to make a significant effect on the chemical composition of the meat. The breast meat CP was reported to be between 23.11% and 25.13% and EE content between 1.69% and 1.84% in Betong chicken (Makchumpon *et al*., 2015; Ritchoo *et al*., 2019). In this study, the CP content was seen to be within that range, but EE was slightly higher, probably due to difference in the age of the birds at slaughter and their diets.

Furthermore, although saturated fatty acid composition of the chicken diets differed significantly, only stearic acid (C18:0) content of the breast meat decreased significantly. The proportions of other saturated, monounsaturated and polyunsaturated fatty acids were not significantly affected by CG. Nevertheless, the saturated fatty acid contents reduced and the unsaturated fatty acid contents increased in the meat of Betong chicken fed with CG. Erol *et al*. (2009) reported significant decline in the stearic acid content in the egg yolk of quails maintained on feeds supplemented with glycerol. Urgnani *et al*. (2014) also described that stearic acid content reduced linearly in the thigh meat of broilers, fed with increasing amounts of crude vegetable glycerin. However, these authors also observed a linear increase in the saturated fatty acids (myristic-, palmitic- and stearic-acids) in chicken fed with semi-purified vegetable glycerin. In contrast, Garcia *et al*. (2018) reported an increasing and decreasing quadratic effect in monounsaturated and polyunsaturated fatty acids, respectively in broilers fed with mixed semi-purified glycerin. The variations in fatty acid profiles in these studies may be related to the sources and levels of CG inclusion in diet and fatty acid profiles of the CG, as well as that of other ingredients of the diet.

In conclusion, the CG can be used at concentrations up to 10% in the diet of Betong chickens at 8 to 20 weeks of age, without any adverse effects on carcass characteristic, chemical composition, and meat quality. However, its effect on breast meat color and lipid oxidation need to be considered.

## Acknowledgments

This work was supported by the Higher Education Research Promotion and Thailand's Education Hub for Southern Region of ASEAN Countries Project Office of the Higher Education Commission (TEH-AC 030/2017). The authors are grateful to Faculty of Natural Resources, Prince of Songkla University for research budget fund (NAT_RF62003). This work was partly supported by Graduate School dissertation fund.

## Conflicts of Interest

The authors declare no conflict of interest.

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