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ANIMAL PRODUCTION

Carcass characteristics and meat quality of broilers fed with crude glycerin originated from palm oil and wasted vegetable oil in diets

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ABSTRACT. A study was conducted to evaluate the effect of crude glycerin on slaughter weight and carcass characteristics of broilers. Total of 180 male Ross - 308 day old chicks were allocated to 3 treatments with 4 replicates in a completely randomized design. The dietary treatments were: diet without crude glycerin inclusion (T1), diet with 5% crude glycerin inclusion from waste vegetable oil (T2), and diet with 5% crude glycerin inclusion from palm oil (T3). On day 42, twenty chickens per treatment were randomly sampled and slaughtered to determine the carcass characteristics. From the study, both crude glycerins did not give any effect on the slaughter weight and carcass characteristics, but broilers received crude glycerin had higher abdominal fat ($p < 0.001$) than control. Drip loss, cooking loss, pH, and meat color were not influenced by both types of crude glycerin, however, the skin color was significantly affected by palm oil crude glycerin inclusion ($p < 0.05$). Furthermore, meat from broilers with crude glycerin had higher lipid oxidation than control ($p < 0.001$). In conclusion, crude glycerins could be added in broiler's diet, however its effect on increasing fat and lipid oxidation must be taken into concern.

Keywords: biodiesel's by product; slaughter weight; lipid oxidation.

Características da carcaça e da qualidade da carne de frango de corte alimentados com glicerina bruta proveniente do óleo de palma e de resíduos de óleo vegetal

RESUMO. Um estudo foi conduzido para avaliar o efeito da glicerina bruta no peso de abate e nas características de carcaça de frangos de corte. Um total de 180 machos da espécie Ross-308 foram alocados em 3 tratamentos com 4 repetições, em um modelo randomizado. Os tratamentos de dieta eram: dieta sem a inclusão da glicerina bruta (T1), dieta com 5% de inclusão de glicerina bruta a partir de resíduos de óleo vegetal (T2), e dieta com 5% de inclusão da glicerina bruta retirada do óleo de palma (T3). No dia 42, vinte aves de cada tratamento foram escolhidas aleatoriamente para serem abatidas e determinar as características da carcaça. A partir do estudo, ambas as glicerinas brutas não afetaram o peso de abate e nem as características na carcaça, porém os frangos que receberam a glicerina bruta tiveram uma maior gordura abdominal ($p < 0.001$). A perda por gotejamento, perda no cozimento, pH, e a coloração da carne não foram influenciadas pelos dois tipos de glicerina, entretanto, a cor da pele foi afetada significantemente pela inclusão da glicerina do óleo de palma ($p < 0.05$). Além disso, a carne dos frangos que tiveram a inclusão da glicerina tiveram uma maior oxidação lipídica ($p < 0.001$). Como conclusão, as glicerinas brutas podem ser adicionadas à dieta de frangos de corte, no entanto, seu efeito no aumento da gordura e da oxidação lipídica deve ser considerados.

Palavras-chave: derivados do biodiesel; peso de abate; oxidação lipídica.

Introduction

Crude glycerin is the main co-product from biodiesel production with low commercial value due to it has contaminants around 20 to 60%, such as, methanol, sodium, potassium, fatty acids, and moisture contents (Min, Yan, Liu, Coto, & Waldroup, 2010). However, it contained 3100 to 6021 GE kcal kg^{-1} or 2535 to 5206 ME kcal kg^{-1} (Kerr, Weber, Dozier III, & Kidd, 2009) which could be mixed in the diets as energy source for livestock. Many studies reported the utilization of crude glycerin as feedstuff in non ruminant like broiler chicken, it can be used up to 10% in the diet without giving any negative effects on growth performance (Jung & Batal, 2011; Min et al., 2010). In addition, it is recommended to be included in the broiler diet about 5.0 to 7.5% due to it gave a positive effect, such as, increasing the feed intake, increasing body weight, and better feed efficiency than diet without crude glycerin (Cerrate et al., 2006; Sehu, Kucukersan, Coskun, Koksal, & Citil, 2012; Silva et al., 2012).

Although, it could give better effect on growth performance and feed efficiency, further study about its effect on meat quality is still lacking. Especially, crude glycerin which derived from palm oil and wasted vegetable oil as the feedstock in biodiesel production which are the mainly feedstocks used in Southeast Asia. Crude glycerin with wasted vegetable oil as feedstock generally has low purity and high content of fat (Kerr, Shurson, Johnston, & Dozier III, 2011), meanwhile crude glycerin from fresh vegetable oil has high purity and low content of fat. The different purity and fat contents between crude glycerins is due to wasted vegetable oil has high contents of free fatty acid and water. Most biodiesel industries uses alkali as its catalyst, however, alkali is susceptible to water and free fatty acid. Free fatty acid may react to form sodium oleate and water, water will cause saponification thus lessen the catalyst effectiveness, furthermore, it will make incomplete transesterification reaction during biodiesel production process. Therefore, this study aimed to compare crude glycerin originated from palm oil which is derived from large scale producer (New Biodiesel Co., Ltd., Surat thani province) and wasted vegetable oil from medium scale producer (R&D Center for Alternative energy Faculty of Engineering, Prince of Songkla University, Songkhla Province) and evaluate its effects on carcass characteristics and meat quality.

Material and methods

This experiment was reviewed and approved by the Institutional Board under the Ethical Principles for the Use of Animals for Scientific Purposes of Prince of Songkla University in Thailand.

Experimental birds and management

A total of 180 Ross 308-day-old male broilers were obtained from local company where they had been vaccinated for Newcastle Disease and Infectious Bronchitis post hatch. The chickens were allocated randomly into 3 feeding treatments each had 4 replications with 15 chickens per replicate pen (1.5 x 2.3 m^2). On week 6, broilers were weighed to determine the slaughter weight and 20 chickens per treatment were selected to determine the carcass characteristics and meat quality.

Experiment was conducted in an evaporative housing system, the temperature of the housing was controlled since the day of the chicken arriving until the slaughtering day. Broilers were provided with 24 hours light per day and had *ad libitum* access to the feed and water.

Diets and experimental procedures

Experimental feeds were based on corn-soybean meal, it was formulated into two feeding phases according to NRC (1994), starter 0 to 3 weeks (23% CP; 3200 kcal kg^{-1} ME per kg) and finisher 4 to 6 weeks (20% CP; 3200 kcal kg^{-1} ME per kg). It was given in mash form. The crude glycerins used in the experiment were obtained from large and medium scale biodiesel producers. Crude glycerins were analyzed according to Sri-muang, Wattanachant, Ngampongsai, and Settapong (2015), crude glycerin with high purity from large scale biodiesel producer had palm oil as feedstock (CGPO) with chemical content 89.49% glycerol and 1.73% crude fat, meanwhile crude glycerin with low purity from medium scale biodiesel producer had wasted vegetable oil as feedstock (CGWVO) with 38.36% glycerol and 23.91% crude fat content. The details of experimental feeds were:

T1: Basal diet (control)

T2: Basal diet with 5% CGWVO from total diet

T3: Basal diet with 5% CGPO from total diet

Metabolizable energy contents were analyzed by Legawa, Wattanasit and Wattanachant (2017), CGPO were 3068.73 kcal kg^{-1} and CGWVO were 4054.52 kcal kg^{-1}. Table 1 shows the feed composition and nutrients content.

Table 1. Experimental diet composition (as fed) and nutrients content (g kg^{-1}).

Feed ingredient	1 to 21 day of age			22 to 42 day of age		
	T1	T2	T3	T1	T2	T3
Corn	569.0	512.0	512.0	631.8	572.8	572.8
Palm oil	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal	272.8	280.8	280.8	210.0	220.0	220.0
Fish meal	85.0	85.0	85.0	85.0	85.0	85.0
Dicalcium phosphate	14.0	14.0	14.0	14.0	14.0	14.0
DL-methionine 99%	2.3	1.3	1.3	2.3	1.3	1.3
L-lysine HCl 78.5%	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin-mineral mix[1]	4.9	4.9	4.9	4.9	4.9	4.9
CGPO	0.0	0.0	50.0	0.0	0.0	50.0
CGWVO	0.0	50.0	0.0	0.0	50.0	0.0
Calculated content						
ME (kcal kg^{-1})	3190	3218	3170	3232	3260	3214
Crude protein	230	230	230	200	200	200
Ether extract	79.9	90.0	78.9	81.1	91.1	80.0
Lysine (digestible)	14.0	14.0	14.0	12.4	12.5	12.5
Methionine (digestible)	6.3	5.3	5.3	6.0	5.0	5.0
Threonine (digestible)	8.4	8.3	8.3	7.5	7.5	7.5
Calcium	10.2	10.2	10.2	10.0	10.0	10.0
Available phosphorus	5.4	5.4	5.4	5.3	5.3	5.3
Sodium	2.1	2.1	2.1	2.1	2.1	2.1
Analyzed content[2]						
Crude protein	229.2	229.3	227.1	199.5	198.9	204.5
Ash	52.6	58.9	53.0	50.1	45.2	48.3
Ether extract	71.4	82.5	68.5	83.5	96.2	89.6

[1]The vitamin-mineral mix provided the following (per kg of diet): cholecalciferol: 320,000 IU; retinol: 2,000,000 IU; tocopheryl: 2,000 mg; thiamin: 220 mg; riboflavin: 450 mg; cyanocobalamin: 4.5 mg; menadione: 330 mg; nicotinic acid: 600 mg; iron: 10,000 mg; copper: 100 mg; manganese: 8,800 mg; iodine: 150 mg; zinc: 8,800 mg; calcium: 52,800 mg; cobalt: 130 mg. [2]Analyzed chemical composition, according to Association Official Analytical Chemist (AOAC, 2006) method

Chemical and physical analysis

On week 6, twenty chickens per treatment were slaughtered to collect the carcass yield, weight of internal organ, and meat samples data. Breast muscle (*Pectoralis major*) was used for physical and chemical analysis. The variables were:

pH

The pH of breast muscles was directly determined by digital pH meter (Seven2Go, Mettler-Toledo, Switzerland) 45 minutes after slaughtered for pH_{45min} and twenty-four hours after slaughter for pH_{24h}.

Drip loss

The breast meat samples were cut into small equal pieces of dimensions of 2.0 × 1.0 × 0.5 cm. Each piece was weighed and packed into a sealed plastic bag before storage at a chilled temperature (4°C). After 24h, the sample was removed from the sealed plastic bag, blotted, and weighed. Percentage of after chilled weight and fresh weight of samples was calculated for drip loss.

Cooking loss

The breast meat samples were cut into small equal pieces of dimensions of 2.0 × 1.0 × 0.5 cm. The samples were put in a plastic-sealed bag and heated in a water bath at 80°C for 10 minutes. After cooking, the sample was cooled until reaching normal temperature. The percentage of sample after and before cooking were calculated as cooking loss.

Texture

The breast samples from cooking loss after cooked were used for texture determination. Texture Analyzer (TA-XT plus Stable Micro System Texture Analyzer, UK) was used to determine the shear force value of the meat using a Warner-Bratzler blade. The operating parameters consisted of a cross-head speed of 2 mm s^{-1} and a 50 kg load cell.

Meat and skin color

Breast meat and breast skin color were determined using a Konica minolta colorimeter (Konica Minolta, Japan) after 24h chilled at 4°C. The colorimeter was calibrated using a black glass and white standard tile before sample analysis. The result was reported in the complete International Commission on Illumination (CIE) system color profile of lightness (L*), redness (a*), and yellowness (b*).

Proximate analysis

The dry matter, ash, crude protein, and ether extract were measured as the standards of the Association of Official Analytical Chemists (AOAC, 2006). Moisture content was analyzed by drying the samples for 24h at 105°C. Crude protein content was analyzed by the Kjeldahl method with destruction, distillation, and titration. Crude fat content was analyzed using soxhlet extraction with diethyl ether. Ash content was analyzed by burning the sample for 3h in the furnace at 550°C.

Thiobarbituric acid value (TBARS)

TBARS number was analyzed based on Buege and Aust (1978) method: 1 g of meat sample was chopped and put into 50 mL centrifuge tube with 5 mL mixture solution containing 15% Trichloroacetic acid (TCA), 0.375% Thiobarbituric acid (TBA), and 0.25N HCl in the same amount ratio. Homogenizer with 13,500 rpm was used for one minute. The mixture was boiled in the boiling water for 10 minutes and cooled by running tap water. The 3,600xg centrifugation was conducted for 20 minutes. Supernatant was placed in the test tube prior to transfer to cuvette. Absorbance was determined using UV-spectrophotometer at 532 nm. Standard curve of malonaldehyde (0 to 100 ppm) was used to calculate the TBA formation in the sample. The result was expressed in mg malonaldehyde per kg of the meat.

Statistical analysis

Data were analyzed for significance by a one-way ANOVA model and further analysis with Tukey's test using computer program SPSS Inc (SPSS, 2007) version 16.

Results and discussion

Slaughter weight, carcass characteristics and the weight of internal organ are presented in Table 2. Crude glycerin inclusion in the diet did not give any effect to slaughter weight.

Slaughtered weight of chicken with crude glycerin was similar to control. Similar results were found by other authors which also did experiment with 5% crude glycerin inclusion in the diets. They concluded that crude glycerin did not affect the body weight of broilers (Cerrate et al., 2006; Silva et al., 2012; Urgnani et al., 2014). In addition, Topal and Ozdogan (2013) found that 4% inclusion of crude glycerin improved weight gain, although it is not significantly different. Carcass, breast, leg quarters, and wing yields were not influenced by inclusion of crude glycerin. Furthermore, weight of

internal organ such as, liver, heart, gizzard, and intestines was also not affected by crude glycerin. However, the abdominal fat of the broilers fed with crude glycerin was higher than control ($p < 0.001$).

Table 2. Slaughter weight (g), carcass characteristics, and internal organ weight (g $100g^{-1}$ of body weight).

Parameters	T1	T2	T3	p-value	SEM
Slaughter weight	2517.31 ± 146.25	2614.81 ± 158.74	2560.56 ± 127.81	0.174	20.903
Carcass yield[1]	73.12 ± 2.15	73.03 ± 2.17	72.60 ± 2.01	0.758	0.315
Breast yield	32.07 ± 1.90	32.59 ± 2.09	31.76 ± 1.92	0.478	0.287
Leg quarters[2]	28.55 ± 1.73	28.42 ± 1.72	28.08 ± 1.35	0.754	0.257
Wing yield	10.73 ± 0.79	10.43 ± 0.77	10.36 ± 0.80	0.329	0.105
Internal organ					
Liver	1.812±0.18	1.783±0.22	1.865±0.25	0.509	0.029
Heart	0.508±0.05	0.506±0.08	0.549±0.07	0.164	0.010
Gizzard	1.009±0.12	1.003±0.09	1.013±0.09	0.971	0.016
Intestine	2.502±0.29	2.412±0.31	2.406±0.25	0.600	0.042
Abdominal fat	1.597±0.08[b]	1.838±0.11[a]	1.826±0.09[a]	0.000	0.019

T1= control diet, T2= diet with 50 g kg^{-1} CGWVO, T3= diet with 50 g kg^{-1} CGPO; [1]Carcass yield is the weight of the chicken after viscerated without internal organs, head, neck, and feet; [2]Leg quarters is the total weight of thigh and drumstick yield; [a,b] Means within the row with different superscripts differ significantly ($p < 0.05$).

Abdominal fat can be a parameter for judging the total body fat in poultry due to it grows faster than another parts of fat tissues (Butterwith, 1989; Fouad, & El-Senousey, 2014). Diet with wasted vegetable oil crude glycerin inclusion in both phases had the highest energy and ether extract among the other experimental feeds. Meanwhile, broiler fed with palm oil crude glycerin diet had higher ether extract content on the grower phase, it was 7.31% than control. Glycerol after absorbed can be converted into glucose via gluconeogenesis, if the blood glucose is high it will be stored as glycogen, furthermore it will be stored as triglyceride in the adipose tissue. Glycerol also can be used for forming triglyceride directly if there are free fatty acids as it is a backbone of triglyceride (Kerr et al., 2011). Those factors maybe the reason of high content on abdominal fat. Silva et al. (2012) observed the abdominal fat linearly increased with 0, 2.5, 5.0, and 7.5 % crude glycerin inclusion but statistically not significant. Lessard, Lefrancois and Bernier (1993) had similar result by 5% inclusion of glycerol, it increased the abdominal fat.

The influences of crude glycerin from different sources and quality on the physical meat quality are shown in Table 3.

Experimental feed with crude glycerin did not affect the physical meat quality, except the color of skin. Both crude glycerin gave higher value of redness and yellowness, but only experimental feed with crude glycerin from large scale which palm oil as the feedstock improved skin pigmentation ($p < 0.05$). This result could not be compared with the previous study because none discussed about the effect of crude glycerin to the skin color of broilers. In this study, the experimental feed with 5% inclusion of crude glycerin had 9.3 to 15.3% lower content of corn, in order to balance energy content of feed. Corn is known to have xanthophylls, the substances that give pigmentation to yolk and skin in poultry. It is responsible for yellow, orange, and red pigmentation (Scott & Eldridge, 2005). However, a fewer corn in the experimental feeds with crude glycerin still had a significant effect to yellowness and redness of the skin. The reason behind this was because of the lutein content in the palm oil which is the feedstock of biodiesel. Ping and Gwendoline (2006) determined lutein and carotenoids of crude palm oil, they identified predominantly α- and ß-carotenes and also minor lutein content. In addition, Tunio et al. (2013) reported that lutein has better pigmentation on broiler's skin, meanwhile, zeaxanthin and canthaxanthin are mainly deposited in broiler fat. Another reason is because of fat content in crude glycerin. Readily digestible fat may improve the utilization of xanthophylls by the chicken (Faulks & Southon, 2005).

Table 3. Physical meat quality of pectoralis major.

Parameters	T1	T2	T3	*P*-value	SEM
pH 45min	6.64 ± 0.13	6.57 ± 0.18	6.55 ± 0.17	0.549	0.033
pH 24h	5.99 ± 0.11	5.98 ± 0.12	5.99 ± 0.09	0.988	0.022
Drip loss (%)	1.302 ± 0.27	1.383 ± 0.38	1.196 ± 0.34	0.367	0.050
Cooking loss (%)	22.61 ± 0.99	22.23 ± 1.13	23.43 ± 1.10	0.103	0.210
Firmness (kg cm^{-2})	1.333 ± 0.36	1.372 ± 0.32	1.338 ± 0.39	0.954	0.055
Meat colour					
L*	51.71 ± 4.87	52.35 ± 3.65	52.02 ± 2.18	0.946	0.793
a*	1.700 ± 0.24	2.067 ± 0.82	2.186 ± 0.85	0.411	0.155
b*	8.73 ± 1.97	8.49 ± 1.16	8.85 ± 1.39	0.909	0.366
Skin colour					
L*	63.60 ± 1.33	63.42 ± 0.73	62.75 ± 1.34	0.384	0.247
a*	2.120 ± 0.67[b]	3.033 ± 1.34[ab]	3.792 ± 0.72[a]	0.012	0.205
b*	11.18 ± 0.62[b]	12.46 ± 1.65[ab]	13.51 ± 1.49[a]	0.007	0.261

T1= control diet, T2= diet with 50 g kg^{-1} CGWVO, T3= diet with 50g kg^{-1} CGPO; L*= lightness, a*= redness, b*= yellowness; [a,b] Means within the row with different superscripts differ significantly ($p < 0.05$).

The only nutrient composition affected by the crude glycerin inclusion was ether extract (Table 4).

Table 4. Nutrient composition of pectoralis major (%).

Parameters	T1	T2	T3	p < value	SEM
Dry matter	25.41 ± 0.44	24.65 ± 0.45	24.59 ± 1.23	0.101	0.163
Ash[1]	1.39 ± 0.08	1.31 ± 0.11	1.27 ± 0.09	0.086	0.021
Ash[2]	5.50 ± 0.32	5.32 ± 0.39	5.14 ± 0.22	0.128	0.069
Crude protein[1]	22.57 ± 0.45	21.62 ± 0.89	21.54 ± 0.93	0.067	0.181
Crude protein[2]	92.01 ± 1.36	90.38 ± 2.99	90.24 ± 2.97	0.326	0.522
Ether extract[2]	2.87 ± 1.32[b]	5.66 ± 1.30[a]	5.69 ± 2.20[a]	0.003	0.340

T1= control diet, T2= diet with 50 g kg^{-1} CGWVO, T3= diet with 50g kg^{-1} CGPO [1]Based on fresh meat; [2]Based on dry matter; [a,b,c]Means within the row with different superscripts differ significantly ($p < 0.05$)

The ether extract content of breast meat was higher for both crude glycerin, while no effect on dry matter, ash, and crude protein. Ether extract in

the experimental feed with crude glycerin inclusion is higher than control which explained the higher crude fat content in the breast muscle ($p < 0.05$). Bogosavljević-Bošković, Pavlovski, Petrović, Dosković and Rakonjac (2010) reported that nutrition is one of the major factors in broiler production. It has a crucial effect on the chemical composition of broiler meat. Higher fat in the diet will result a higher fat content of the meat and fat deposit in adipose tissue. At contrary finding from Topal and Ozdogan (2013) which the ether extract in drumstick muscle was decreasing in broilers fed with 4% crude glycerin inclusion diet. This was due to the decreasing levels of ether extract in the diet with glycerin inclusion. However, ether extract in the experimental feed with crude glycerin inclusion was higher than control.

The oxidative stability of meat samples was analyzed using thiobaribituric acid reactive substances (TBARS) method. TBARS numbers in this study were determined after 3 day keeping in the 4°C at chilling room (Figure 1).

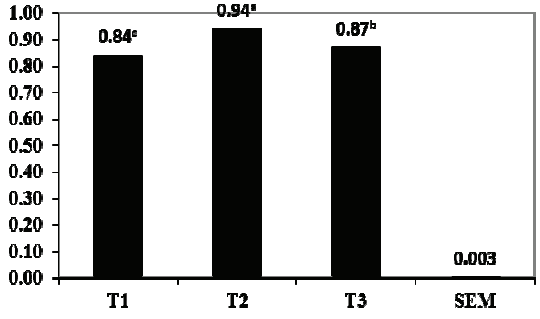

Figure 1. Thiobarbituric acid reactive substances of breast meat samples (mg malonaldehyde kg^{-1}). P-value = 0.000; SEM = 0.003. T1 = control diet, T2 = diet with 50 g kg^{-1} CGWVO, T3 = diet with 50 g kg^{-1} CGPO.

TBARS values of meat with 5% crude glycerin inclusion was greater than control ($p < 0.001$) and with inclusion of diet with wasted vegetable oil crude glycerin (T2) had the highest value. TBARS value is influenced by the amount of fat on meat. Meat samples with crude glycerin inclusion had higher ether extract content compare to control which explained the greater TBARS value. Study by Hugo, Els, De Witt, Van Der Merwe and Fair (2009) which observe the effect of dietary lipid sources to the lipid oxidation in broiler meat, confirmed that the higher inclusion of lipid sources will increase the fat oxidation during refrigerated storage for meat sample. Antioxidant such as vitamin E is crucial to use if crude glycerin is added into diet.

Conclusion

It can be concluded that 5% crude glycerin inclusion in the diet did not give any negative effect to slaughtered weight, carcass characteristics, and meat quality. However, it increased the abdominal fat and ether extract content of meat. Furthermore, it increases the lipid oxidation, especially crude glycerin from wasted vegetable oil. Palm oil crude glycerin improved the redness and yellowness of broiler's skin. Both crude glycerins are applicable to be included in the broiler's diet, nevertheless, its effect on increasing the lipid oxidation must be taken into concern.

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