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Growth performance, abdominal fat and fat digestibility in broiler chicken fed with synthetic emulsifier and natural biosurfactant

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ABSTRACT

An experiment was conducted to evaluate the response of exogenous emulsifier on broiler performance, abdominal fat and fat digestibility. A corn-soy based broiler diet was formulated with fixed ratio 2:1 (oil-to-water), supplemented with glyceryl polyethylene glycol ricinoleate (GPGR) synthetic emulsifier and lysophosphatidylcholine (LPC) natural biosurfactant. One-day-old Cobb 500 male broilers (1,800) with nine treatments diet were used in this study. The treatments were T1, Basal diet with metabolizable energy (ME) 3,000 kcal/kg in starter (S) and 3,100 kcal/kg in grower (G); T2, Basal diet with ME 2,900 kcal/kg (S) and 3,000 kcal/kg (G); T3, Basal diet with ME 2,800 kcal/kg (S) and 2,900 kcal/kg (G); T4, T5 and T6 consisted of T1, T2 and T3 supplemented with GPGR; T7, T8 and T9 consisted of T1, T2 and T3 supplemented with LPC. The study was conducted 14 days for starter and another 21 days for grower phase. The results of the experiment demonstrated the effect of emulsifier on broiler performance was dependent on the ME level used in the diet formulations and ages of the bird. Emulsifier improved FCR in starter phase at higher ME level, but was not significantly improved at lower ME levels of diets. Correlation between emulsifier and low ME diet in FCR was not observed in present study. Fat digestibility at all levels of ME were higher in birds fed with emulsified diets. However, compensatory effect to recover the energy value to control level was not found. Significant reduction in abdominal fat and digesta fat was observed in birds which consumed emulsified grower diets. However, significant difference between synthetic emulsifier and natural biosurfactant was not observed.

Key words: Digesta, Emulsifier, Fat digestibility, Feed conversion ratio, Metabolizable energy

A variety of lipid supplements are available in current poultry industry. As a consequence of the country resources, crude palm oil is widely used in this region as the main energy source in poultry diet. However, crude palm oil contains a high proportion of saturated fatty acid and possesses relatively low ME value as calculated using Wiseman's equation (Walker 2011). A synergy with some dose effect was reported between saturated and unsaturated fat in broiler chicken. A limiting digestibility was reported for long-chain saturated fat, but moderate proportion of saturated lipid or relative lower unsaturated: saturated fatty acid ratio was reported to have better utilization of dietary fat in poultry (Wiseman *et al*. 1998). In young poultry, the physiological limitation has reduced the availability of secreted bile salts and lipase in gastrointestinal tract (Noy and Sklan 1995), whereby the formation of micelle by fat emulsification and optimization of lipase activity could not be achieved (Lesson and Atteh 1995). Inclusion of emulsifying agent has been used alternatively to increase the fat digestion and nutrient absorption in young poultry (Al-Marzooqi and Leeson 1999). In this experiment, synthetic emulsifier glyceryl polyethylene glycol ricinoleate and natural bio surfactant lysophosphatidylcholine were supplemented in the diet with crude palm oil as the main dietary energy source. Different levels of ME were formulated in the experimental diets with fixed inclusion of emulsifier. Effect of emulsifier on energy compensatory response to broiler growth performance, fat digestibility and the respective AME values was studied. It was hypothesized that exogenous emulsifier would enhance the fat digestion by facilitating the process of emulsification.

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MATERIALS AND METHODS

Treatment, diet and design: One-day-old Cobb 500 male broilers (1,800) were used. The study was conducted in 2×3 factorial arrangements with nine treatments, evaluating two types of emulsifiers and three levels of ME. Each treatment was replicated five times with each replicate consisting of 40 birds per pen. All diets were produced by a commercial feed mill. Corn-soy based diet was formulated by least-cost Brill feed formulation software (Feed Management

System 2005) meeting the nutritional recommendations of Cobb 500 (Tables 1, 2). A 2-phase feeding program was adopted; starter feed in crumble form (1 to 14 days) and grower feed in pellet form (15 to 35 days). Treatment 1 consisted of basal diet with ME 3,000 kcal/kg and 3,100 kcal/kg in starter and grower phase, respectively; Treatment 2 consisted of basal diet with 100 kcal/kg reduction in ME; Treatment 3 consisted of basal diet with 200 kcal/kg reduction in ME; Treatments 4, 5 and 6 consisted of Treatments 1, 2 and 3 supplemented with glyceryl polyethylene glycol ricinoleate; Treatments 7, 8 and 9 consisted of Treatments 1, 2 and 3 supplemented with lysophosphatidylcholine as comparative treatment. All diets were isocaloric,isonitrogenous and contained similar digestible amino acid percentage with formulation having the same ingredient profile. Glycerol polyethylene glycol ricinoleate, a product suggested to be used in milling process to improve the manufacturing efficiency through water-oil emulsification was introduced into this experiment. The effect of this synthetic emulsifier to extend its functionality up to the gastrointestinal tract was determined. In order to have the same treatment condition, all the experiment diets were formulated with 2% oil added at milling mixer, with the remaining of the oil inclusion as formulated in the diet

Table 1. Ingredients and calculated nutrient of starter diet formulation

Ingredient	Unit	Starter diet, 1-14 days								
		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Corn	%	43.921	43.929	43.888	43.831	43.945	43.904	43.871	43.948	43.908
Soybean meal (46%)	%	42.271	42.271	42.271	42.271	42.271	42.271	42.271	42.271	42.271
Crude palm oil, added at mixer	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Crude palm oil, added after pelleting	%	4.450	2.400	0.400	4.500	2.400	0.400	4.450	2.400	0.400
Rice bran	%	2.190	2.190	2.190	2.190	2.190	2.190	2.190	2.190	2.190
Corn gluten meal (60%)	%	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Di-calcium phosphate (18%)	%	1.784	1.784	1.784	1.784	1.784	1.784	1.784	1.784	1.784
Limestone	%	1.109	1.109	1.109	1.109	1.109	1.109	1.109	1.109	1.109
Salt	%	0.310	0.310	0.310	0.310	0.310	0.310	0.310	0.310	0.310
DL-methionine	%	0.308	0.308	0.308	0.308	0.308	0.308	0.308	0.308	0.308
[1]Premix	%	0.800	0.800	0.800	0.800	0.800	0.800	0.800	0.800	0.800
Sodium bicarbonate	%	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220
Lysine	%	0.070	0.070	0.070	0.070	0.070	0.070	0.070	0.070	0.070
Choline chloride (75%)	%	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Threonine	%	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
[2]Synthetic emulsifier	%	-	-	-	0.050	0.050	0.050	-	-	-
[3]Natural biosurfactant	%	-	-	-	-	-	-	0.050	0.050	0.050
Water	%	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Wheat pollard	%	-	2.042	4.082	-	1.986	4.027	-	1.972	4.013
					Calculated nutrient					
ME	kcal/kg	3,000	2,900	2,800	3,000	2,900	2,800	3,000	2,900	2,800
Crude protein	%	23.69	24.00	24.30	23.68	23.99	24.29	23.69	23.99	24.29
Crude fat	%	9.07	7.09	5.17	9.12	7.09	5.16	9.07	7.09	5.16
Calcium	%	0.99	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00
Available phosphorus	%	0.48	0.49	0.49	0.48	0.49	0.49	0.48	0.49	0.49
Dig lysine	%	1.28	1.29	1.30	1.28	1.29	1.30	1.28	1.29	1.30
Dig methionine	%	0.63	0.63	0.64	0.63	0.63	0.64	0.63	0.63	0.64
Dig threonine	%	0.81	0.82	0.82	0.81	0.82	0.82	0.81	0.82	0.82

[1]Supplied per kg of diet: Fe, 40 mg; Zn, 100 mg; Mn, 120 mg; Cu, 20 mg; Se, 0.3 mg; vitamin A, 12 kIU; vitamin D, 4.4 kIU; vitamin E, 57 mg; vitamin K, 2.8 mg; vitamin B_1, 3.06 mg; vitamin B_2, 6.72 mg; vitamin B_6, 5.49 mg; vitamin B_{12}, 0.028 mg; niacin, 67.3 mg; folic acid, 1.33 mg; pantothenic acid, 14.8 mg; biotin, 0.26 mg; ethoxyquin, 100 mg; growth promoter, 140 mg; anticoccidial, 200 mg; mold inhibitor, 500 mg. [2]Each 1 kg contained 100% glyceryl polyethylene glycol ricinoleate. [3]Each 1 kg contains 250 g lysophosphatidylcholine.

Table 2. Ingredients and calculated nutrient of grower diet formulation

Ingredient	Unit	Grower diet, 15-35 days								
		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Corn	%	52.514	52.520	52.520	52.226	52.520	52.520	52.226	52.520	52.520
Soybean meal (46%)	%	32.297	32.297	32.297	32.297	32.297	32.297	32.297	32.297	32.297
Crude palm oil, added at mixer	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Crude palm oil, added after pelleting	%	3.293	1.567	-	3.381	1.584	-	3.381	1.584	-
Rice bran	%	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Corn gluten meal (60%)	%	0.613	0.613	0.613	0.613	0.613	0.613	0.613	0.613	0.613
Di-calcium phosphate (18%)	%	1.639	1.639	1.639	1.639	1.639	1.639	1.639	1.639	1.639
Limestone	%	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889
Salt	%	0.310	0.310	0.310	0.310	0.310	0.310	0.310	0.310	0.310
DL-methionine	%	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246	0.246
[1]Premix	%	0.800	0.800	0.800	0.800	0.800	0.800	0.800	0.800	0.800
Sodium bicarbonate	%	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240
Lysine	%	0.082	0.082	0.082	0.082	0.082	0.082	0.082	0.082	0.082
Choline chloride (75%)	%	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Threonine	%	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026
[2]Synthetic emulsifier	%	-	-	-	0.050	0.050	0.050	-	-	-
[3]Natural biosurfactant	%	-	-	-	-	-	-	0.050	0.050	0.050
Water	%	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Wheat pollard	%	0.003	1.723	3.290	0.153	1.656	3.240	0.153	1.656	3.240
Calculated nutrient										
ME	kcal/kg	3,100	3,000	2,900	3,100	3,000	2,900	3,100	3,000	2,900
Crude protein	%	20.16	20.42	20.65	20.16	20.41	20.64	20.16	20.41	20.64
Crude fat	%	8.53	6.87	5.36	8.62	6.88	5.36	8.62	6.88	5.36
Calcium	%	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Available phosphorus	%	0.44	0.45	0.46	0.44	0.45	0.46	0.44	0.45	0.46
Dig lysine	%	1.06	1.07	1.08	1.06	1.07	1.07	1.06	1.07	1.07
Dig methionine	%	0.53	0.53	0.54	0.53	0.53	0.54	0.53	0.53	0.54
Dig threonine	%	0.69	0.70	0.70	0.69	0.70	0.70	0.69	0.70	0.70

[1]Supplied per kg of diet: Fe, 40 mg; Zn, 100 mg; Mn, 120 mg; Cu, 20 mg; Se, 0.3 mg; vitamin A, 10.5 kIU; vitamin D, 3.8 kIU; vitamin E, 50 mg; vitamin K, 2.45 mg; vitamin B_1, 2.68 mg; vitamin B_2, 5.88 mg; vitamin B_6, 4.8 mg; vitamin B_{12}, 0.025 mg; niacin, 58.9 mg; folic acid, 1.16 mg; pantothenic acid, 12.9 mg; biotin, 0.23 mg; ethoxyquin, 100 mg; growth promoter, 125 mg; anticoccidial, 550 mg; mold inhibitor, 500 mg. [2]Each 1 kg contains 100% glyceryl polyethylene glycol ricinoleate. [3]Each 1 kg contains 250 g lysophosphatidylcholine.

added through post-pelleting spray. Water (1%) was added directly into the mixer through atomize nozzle for treatments 1, 2, 3, 7, 8 and 9. Glyceryl polyethylene glycol ricinoleate for treatments 4, 5 and 6 was dispersed into 2% oil phase and 1% water forming an emulsion mixture. This mixture was pre-blended at 60°C for 3 min in an emulsitron system to form water-in-oil (w/o) emulsion before being injected into the mixer. No adjustment of nutrient density for feed treated with water was done. All diets were produced within a day for each feed phase, to eliminate the possible variables associated with feed manufacture. The grower diets were manufactured two weeks after the starter diets.

Farm and facilities: The experiment was carried out at Broga Farm, Selangor. All the birds were vaccinated at hatchery against infectious bursal disease and Newcastle disease. Vaccination of Newcastle disease was introduced in drinking water on the 14^{th} day of age. The birds were floor reared in a curtain-sided positive pressure ventilated close house. This area consisted of a total of forty five floor pens with 2×2 meter square per pen, with each pen equipped with tray feeders and nipple drinkers. Brooding, house temperature and ventilation program was controlled to maintain broiler comfort temperature. Twenty four hours lighting program was adopted. Feed and water were provided *ad-lib.* throughout the entire experiment period. The experimental animals received humane care as outlined and approved by Institutional Animal Care and Use Committee for the Care and Use of Animals for Scientific Purposes (Research Policy, Universiti Putra Malaysia).

Measurements and analytical methods: Bird performance was evaluated for the cumulative period of 14 and 35 days of age. Body weight was obtained by weighing all the birds in each experimental unit on 14 and 35 days of age. Weight gain was calculated by subtracting body weight at each period with the initial body weight. Feed intake was calculated by the difference between the total amount of feed supply and feed residues at the end of each period. Feed conversion ratio (FCR) was calculated as the ratio between total feed supply and weight gain in each period. Mortality was recorded daily. Corrected FCR was calculated by subtracting from the mortality rate. Two uniform sizes of birds were selected from each replicate at day 7 and 28. The birds were adapted in metabolize cage for three days with respective treatment diets and water. Titanium dioxide was added to the diets for another four days as indigestible marker at 5 g/kg (Short *et al.* 1995). All the birds were weighed individually and slaughtered by cervical dislocation on day 14 and 35 for starter and grower phase, respectively. Abdominal fat was collected and weighed relative to live body weights. Breast meat including breast fillet and tender were minced and mixed thoroughly until a subsample size was obtained before meat analysis. Breast meat and liver were extracted for crude fat content by Soxhlet system (AOAC 2006). Ileal digesta was collected between Meckel's diverticulum and the ileo-cecal junction. The digesta was pre-dried at 55°C for 72 h, ground to pass through 0.5 mm sieve before proceeding for nutrient analysis. Moisture content of digesta was determined by thermal drying method (AOAC 2011), which measured the loss in mass under specified condition at 135°C for 2 h. The same samples were extracted for crude fat content by Soxhlet method (AOAC 2006). All the experimental diets and digesta were analyzed for gross energy (ISO 1998), and the concentration of titanium dietary marker was determined as reported by Short *et al*. (1995). Gross energy (GE) was determined by measuring the enthalpy of combustion between benzoic acid and the samples in the bomb calorimeter. The concentration of titanium dioxide was measured by ashing and dissolving the sample in 7.4 M sulphuric acid and hydrogen peroxide (30% vol.). The color intensity of titanium dioxide concentration was determined by obtaining the absorbance at 410 nm directly from UV spectrophotometer. Apparent metabolizable energy (AME) and fat digestibility were calculated and corrected with the recovery factor of indigestible indicator of inert marker as formula below.

$$\text{AME} = \text{GE}_{\text{diet}} - \left[\text{GE}_{\text{digesta}} \times \frac{\text{marker diet}}{\text{marker digesta}}\right]$$

Apparent fat digestibility (%)

$$= 100 - \left[100 \times \frac{\%\ \text{marker diet}}{\%\ \text{marker digesta}} \times \frac{\%\ \text{fat indigesta}}{\%\ \text{fat in diet}}\right]$$

Statistical analysis: All data from the experiments were calculated using General Linear Model Procedure (GLM) of the Statistical Analysis System (SAS Institute 2000). Significant differences were analyzed statistically using Least Significant Different (LSD). Treatment means were compared by Duncan's Multiple Range Test using SAS program. Factorial analysis at different levels of metabolizable energy and types of emulsifier was run to explore the main effects and their interaction. The statistical model used was

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where, Y_{ij}, observed dependent variables; μ, treatment mean; α_i, main effect of metabolizable energy level; β, main effect of types of emulsifier; $(\alpha\beta)_{ij}$, interaction between metabolizable energy and emulsifier; $\varepsilon_{ij,}$ experimental error. Statement of statistical significance was based on probability P< 0.05, and high significance at P<0.0001.

RESULTS AND DISCUSSION

Bird performance, body weight, feed intake and FCR: Body weight gain and feed intake were not affected by dietary treatments in starter phase (d1 to 14) and grower phase (d15 to 35) (Table 3). No significant interactions were observed between types of emulsifier and levels of ME. However, significant improvement (P<0.05) in FCR was observed during starter phase for diets supplemented with lysophosphatidylcholine. FCR was significantly reduced (P<0.05) especially at ME level of 3,000–3,100 kcal/kg and 2,900–3,000 kcal/kg. Although no significant differences was observed in FCR during grower phase, main effect for level of ME was significantly (P<0.05) affected in FCR for overall performance (d1 to 35).

In most practical feeding, major factor that influences feed intake is the energy level of the diet. Birds which consumed lower energy diets had a reduction in body weight and increased feed intake. This was due to the energy requirement of the bird, and the ability of feed intake adjustment for their growth and metabolism needs. Birds adjust their feed intake to maintain a constant energy intake regardless of energy level. Pesti and Smith (1984) reported the broiler chicken response to protein and energy levels in the diet, but not to the energy-to-protein ratio. Since the experimental diets were formulated based on digestible amino acid, the growth difference was due to the energy level rather than the availability of amino acid. Birds fed with higher energy level diets showed better growth and utilized feed efficiently.

Effect of bird performance on energy recovered by emulsifier was observed on day 14. Birds that received diets with emulsifier performed better in feed conversion ratio than those that received control diets at ME level of 3,000–3,100 kcal/kg. Studipto and Ghosh (2010) reported that FCR can be reduced by lowering the feed intake on low energy diet supplemented with nutritional emulsifier. The effect was observed (P<0.05) in lysophosphatidylcholine supplemented diet (T8) at ME level of 2,900–3,000 kcal/kg. The results were in agreement with the findings of Polin *et al*. (1980), who reported that fat absorption in young chick fed on emulsified diet significantly improved in 1 to 3 weeks of age. The performance improvement could be due to the energy that recovered because of the emulsifier. Effect of emulsifier in young broiler up to 21 days could be due to the physiological factor and limitation function in digestive system on fat digestion and nutrient absorption (Jeason and Kellog 1992). Immature digestive tract results

Table 3. Treatment effects on live body weight gain, feed intake and feed conversion ratio

Treatment	ME[3], kcal/kg starter-grower	Mean±SE								
		Body weight gain, kg			Feed intake, kg			FCR[4]		
		Day 1-14	Day 15-35	Day 1-35	Day 1-14	Day 15-35	Day 1-35	Day 1-14	Day 15-35	Day 1-35
T1 : Control	3,000–3,100	0.473± 0.01	1.883± 0.03	2.356± 0.03	0.513± 0.02	3.187± 0.07	3.699± 0.07	1.09± 0.03[a]	1.69± 0.03	1.57± 0.03
T2 : Control	2,900–3,000	0.473± 0.01	1.864± 0.05	2.338± 0.06	0.496± 0.01	3.197± 0.05	3.694± 0.05	1.05± 0.02[ab]	1.72± 0.03	1.58± 0.02
T3 : Control	2,800–2,900	0.468± 0.01	1.782± 0.05	2.251± 0.05	0.495± 0.01	3.136± 0.05	3.631± 0.05	1.06± 0.02[ab]	1.76± 0.04	1.62± 0.03
T4 : Syn. emulsifier[1]	3,000–3,100	0.483± 0.01	1.848± 0.05	2.332± 0.05	0.491± 0.01	3.157± 0.08	3.648± 0.08	1.02± 0.01[b]	1.71± 0.03	1.57± 0.02
T5 : Syn. emulsifier[1]	2,900–3,000	0.478± 0.01	1.840± 0.10	2.317± 0.03	0.499± 0.02	3.181± 0.04	3.680± 0.05	1.04± 0.02[ab]	1.73± 0.02	1.59± 0.02
T6 : Syn. emulsifier[1]	2,800–2,900	0.475± 0.01	1.804± 0.03	2.279± 0.03	0.497± 0.01	3.138± 0.04	3.635± 0.04	1.05± 0.01[ab]	1.74± 0.01	1.60± 0.01
T7 : Nat. biosurfactant[2]	3,000–3,100	0.485± 0.01	1.851± 0.02	2.336± 0.02	0.478± 0.01	3.108± 0.04	3.587± 0.05	0.99± 0.02[c]	1.68± 0.02	1.54± 0.02
T8 : Nat. biosurfactant[2]	2,900–3,000	0.479± 0.01	1.880± 0.02	2.359± 0.03	0.483± 0.01	3.215± 0.05	3.698± 0.06	1.01± 0.02[bc]	1.71± 0.02	1.57± 0.01
T9 : Nat. biosurfactant[2]	2,800–2,900	0.474± 0.01	1.861± 0.03	2.335± 0.03	0.502± 0.02	3.187± 0.06	3.689± 0.07	1.06± 0.03[ab]	1.71± 0.01	1.58± 0.01
P-value		0.6324	0.6163	0.5646	0.3780	0.9376	0.9139	0.0105	0.3644	0.1978
SEM		0.0022	0.0121	0.0125	0.0037	0.0174	0.0187	0.0075	0.0078	0.0064
		Main effect and interaction								
Emulsifier		0.2191	0.5573	0.5147	0.2326	0.9497	0.9115	0.0083	0.3369	0.1448
ME level		0.3088	0.2528	0.1864	0.7728	0.5463	0.6270	0.1928	0.0762	0.0402
Emulsifier × ME level		0.9696	0.6923	0.7491	0.2760	0.8283	0.7281	0.0585	0.8617	0.9449

[a-c]Means within a column with different superscripts differ significantly (P<0.05). SE, Standard error; SEM, Standard error of mean. [1]Each 1 kg contained 100% glyceryl polyethylene glycol ricinoleate. [2]Each 1 kg contained 250 g lysophosphatidylcholine. [3]Metabolizable energy, [4]Feed conversion ratio.

in reduced bile salts secretion which then limits the availability on micelles formation. Lower lipase activity further reduced the fat utilization by young chicks. It was reported that limitation of bile secretion in young broiler gradually increased 8 to 10 folds, and lipase secretion increased 20 to 100 folds between day-4 to day-21 (Noy and Sklan 1995). Lipase activity was reported to reach to the secretion peak only after 40 to 56 days of age (Krogdahl and Sell 1989). The lower apparent metabolize energy in young birds was mainly due to limitation of emulsification effect rather than deficiency in lipase activity (Wiseman 1990). However, the finding of this study contradicted with past research done by Guerreiro *et al*. (2011), who reported that there were no differences in performance on 7-day-old young broiler when fed with emulsified diet. A possible explanation could be that soybean oil was used in that study as a dietary fat compared to crude palm oil used in the present study. Soybean oil has U:S ratio (unsaturated: saturated fatty acid ratio) of 5.7 compared with crude palm oil of 0.95 (Wiseman *et al*. 1998). Palm oil is relatively saturated with high proportions of palmitic acid (C16:0) and stearic acid (C18:0), and it is unlikely to form micelles in the presence of bile salt. The effect of exogenous emulsifier on saturated fatty acid provides a bigger energy compensation gap compared to higher U:S ratio oil sources. Moreover, dietary saturated fat has a greater suppression effect in fatty acid synthesis than unsaturated fat (Waterman *et al*. 1975). It was explained that the activities of acetyl-CoA carboxylase and fatty acid synthetase are inhibited by the dietary saturated fat, whereas the quantity of lipogenic enzymes in the liver is controlled by the polyunsaturated fat (Pearce 1983). However, effect of emulsifier was not observed in broiler performance after 21 days of age in present study. The results coroborrated with the findings of Guerreiro *et al*. (2011) who reported a significant positive effect of emulsified diet on 14-day-old broiler, but no influence on broiler performance at 42 days of age. A similar finding was reported by Zhang *et al*. (2011) where significant weight gain was observed during 1 to 21 days when broilers were started on diet supplemented with lysophosphatidylcholine, but no significant improvement over 1 to 35 days of trial period. The effect of emulsifier in

Table 4. Treatment effects on abdominal fat relative to live body weight, fat content of digesta, liver and breast meat on day 14 and 35

Treatment	ME[3], kcal/kg starter-grower	Mean±SE (%)							
		Day 14				Day 35			
		Abdominal fat	Digesta _fat[4]	Liver _fat[4]	Breast meat _fat[4]	Abdominal fat	Digesta _fat[4]	Liver _fat[4]	Breast meat _fat[4]
T1 : Control	3,000–3,100	1.05± 0.09[ab]	5.75± 0.37[a]	11.83± 0.66	1.37± 0.14	1.90± 0.10[a]	4.55± 0.54[ab]	13.85± 0.98	2.84± 0.40[bc]
T2 : Control	2,900–3,000	1.13± 0.05[a]	3.33± 0.04[c]	11.19± 0.27	1.13± 0.10	1.52± 0.08[abc]	3.96± 0.72[ab]	13.49± 0.52	2.83± 0.25[bc]
T3 : Control	2,800–2,900	0.74± 0.16[bc]	2.20± 0.14[d]	13.05± 0.75	1.28± 0.08	1.43± 0.12[bc]	2.51± 0.04[cd]	13.14± 1.03	2.53± 0.22[c]
T4 : Syn. emulsifier[1]	3,000–3,100	0.64± 0.12[c]	6.11± 0.16[a]	11.48± 0.45	1.64± 0.16	1.79± 0.15[ab]	5.06± 0.11[a]	13.66± 0.52	3.80± 0.42[ab]
T5 : Syn. emulsifier[1]	2,900–3,000	0.78± 0.10[bc]	4.43± 0.32[b]	11.93± 0.47	1.37± 0.10	1.62± 0.12[ab]	4.50± 0.42[ab]	13.00± 0.67	3.75± 0.47[ab]
T6 : Syn. emulsifier[1]	2,800–2,900	0.64± 0.10[c]	3.30± 0.67[c]	11.76± 0.58	1.48± 0.15	1.43± 0.17[bc]	2.46± 0.02[cd]	12.55± 0.84	3.28± 0.30[abc]
T7 : Nat. biosurfactant[2]	3,000–3,100	0.79± 0.14[bc]	4.23± 0.40[bc]	11.75± 0.62	1.31± 0.18	1.55± 0.12[abc]	4.13± 0.32[ab]	12.72± 0.69	3.86± 0.25[a]
T8 : Nat. biosurfactant[2]	2,900–3,000	0.78± 0.11[bc]	3.53± 0.27[bc]	12.04± 0.63	1.29± 0.10	1.40± 0.11[bc]	3.30± 0.15[bc]	11.36± 0.67	3.63± 0.18[ab]
T9 : Nat. biosurfactant[2]	2,800–2,900	0.93± 0.07[abc]	2.08± 0.09[d]	12.56± 0.41	1.29± 0.14	1.16± 0.11[c]	1.85± 0.26[d]	11.97± 0.60	3.46± 0.24[abc]
P-value		0.0155	< .0001	0.4277	0.3013	0.0039	< .0001	0.0496	0.0168
SEM		0.04	0.27	0.18	0.04	0.05	0.26	0.25	0.11
		Main effect and interaction							
Emulsifier		0.1021	0.0006	0.3790	0.0807	0.0327	0.0013	0.0701	0.8669
ME level		0.7911	< .0001	0.5969	0.5768	0.0297	< .0001	0.2807	0.3163
Emulsifier × ME level		0.4017	0.3660	0.8078	0.6705	0.9747	0.5255	0.7428	0.8811

[a-d]Means within a column with different superscripts differ significantly (P<0.05). SE, Standard error; SEM, Standard error of mean. [1]Each 1 kg contained 100% glyceryl polyethylene glycol ricinoleate. [2]Each 1 kg contained 250 g lysophosphatidylcholine. [3]Metabolizable energy, [4]Expressed as dry matter.

energy compensation was not significant in older age broiler, which might be due to the increase of pancreatic lipase (Guerreiro *et al*. 2011) as well as the maturity of enterohepatic circulation (Jeason and Kellog 1992). The gastrointestinal tract is well developed at grower phase and it is able to support the physiological need of fat digestion and absorption.

Although the positive effect of emulsifier was observed in broiler fed on diets with ME level of 2,900–3,000 kcal/kg (T8), correlation effect was not observed at ME level of 2,800–2,900 (T6 and T9). Both synthetic emulsifier and biosurfactant had no significant difference (P>0.05) at this ME level in body weight gain, feed intake and FCR. The result showed an energy-dependent effect between emulsifier and the dietary energy level. The recovery of energy by emulsifier was not found in lower energy diets. Similar finding was reported by Cho *et al*. (2012), who have observed no compensation effect from nutritional emulsifier on low energy broiler diet. From his study, a reduction of 150 kcal/kg metabolism energy on diet with 3% tallow as dietary energy source, and an inclusion of emulsifier sodium stearoyl-2-lactylate showed no significant improvement in bird performance, body weight gain, feed intake, and feed conversion ratio. Positive response was not observed in both starter and grower birds fed on low energy diets which might be due to the limitation of lipase activity in the bird. Krogdahl and Sell (1989) reported that intestinal lipase activity is dependent on dietary fat level, whereby lower level of dietary fat led to lower lipase activity. Although emulsification of fat droplets was achieved to some extent, no significant response in low energy diets was recorded. This could be due to limitation of lipase in fat digestion and utilization. Therefore, to have a significant compensatory effect from exogenous emulsifier, a maximum level of ME reduction in feed formulation needs to be studied.

Abdominal fat, fat content of digesta, liver and breast meat: The abdominal fat relative to live body weights of birds on the 14th and 35th day are represented in Table 4. Significantly lower (P<0.05) abdominal fat was observed in birds that consumed diets with emulsifier than those with control diets regardless of any ME level. A reduction in abdominal fat was observed in birds fed with lower ME level of grower diets. However, two way interactions effect

Table 5. Treatment effects on apparent fat digestibility and apparent metabolizable energy (AME) for starter and grower phase

Treatment	ME[3], kcal/kg starter-grower	Mean±SE			
		Day 14		Day 35	
		Fat digestibility (%)	AME	Fat digestibility (%)	AME
T1 : Control	3,000–3,100	42.56±1.28[c]	2946±1.45[a]	52.20±3.69[cd]	2993±21.43[ab]
T2 : Control	2,900–3,000	58.37±1.22[ab]	2812±8.76[b]	53.12±2.81[bcd]	2946±23.84[bc]
T3 : Control	2,800–2,900	59.60±2.02[ab]	2745±4.04[c]	68.84±1.90[a]	2834±11.05[d]
T4 : Syn. emulsifier[1]	3,000–3,100	41.59±3.07[c]	2923±10.41[a]	49.21±3.80[d]	2985±35.36[ab]
T5 : Syn. emulsifier[1]	2,900–3,000	43.79±1.21[c]	2803±6.67[b]	50.48±2.53[d]	2928±37.99[bc]
T6 : Syn. emulsifier[1]	2,800–2,900	49.79±8.69[bc]	2667±22.81[d]	58.66±2.51[b]	2835±13.38[d]
T7 : Nat. biosurfactant[2]	3,000–3,100	59.54±0.35[ab]	2956±14.73[a]	58.04±2.26[bc]	3034±4.48[a]
T8 : Nat. biosurfactant[2]	2,900–3,000	56.72±1.25[ab]	2827±12.99[b]	57.96±1.91[bc]	2950±3.00[bc]
T9 : Nat. biosurfactant[2]	2,800–2,900	61.69±2.48[a]	2740±24.36[c]	66.47±1.85[a]	2884±2.60[cd]
P-value		0.0005	< .0001	< .0001	< .0001
SEM		1.78	18.95	1.47	14.17
		Main effect and interaction			
Emulsifier		0.0075	0.0115	0.0007	0.0654
ME level		0.3797	< .0001	0.0022	0.0003
Emulsifier × ME level		0.4165	0.3614	0.9425	0.8065

[a-d]Means within a column with different superscripts differ significantly (P<0.05). SE, Standard error; SEM, Standard error of mean. [1]Each 1 kg contained 100% glyceryl polyethylene glycol ricinoleate. [2]Each 1 kg contained 250 g lysophosphatidylcholine. [3]Metabolizable energy.

between emulsifier and ME level in abdominal fat deposition was not observed at any feeding period. Fat content of digesta, liver and breast meat was analyzed and expressed as dry matter (Table 4). Digesta fat was significantly reduced (P<0.0001) in birds which consumed low ME diets (T2, T3, T5, T6, T8 and T9). A significant treatment effect was observed for types of emulsifier (P<0.05) and different levels of ME (P<0.0001). Similar findings were found in grower phase. Birds that consumed diets with emulsifier had no significant difference in liver fat, but possessed higher meat fat (P<0.05) regardless of ME level in grower phase.

Abdominal fat deposition was found to increase with age and level of dietary fat in the diet. Abdominal fat decreased with the decreasing energy level at grower stage (P<0.05). Direct correlation between dietary energy level on abdominal fat and total body fat deposition in grower period was reported by other researches (Kassim and Suwanpradit 1996, Rabie and Szilagyi 1998). Deposition of abdominal fat was reported to increase with the increasing level of dietary fat in birds fed with tallow (Sanz *et al*. 1999). Crespo and Esteve-Garcia (2001) reported that abdominal fat content could be influenced by the fatty acid profile of the dietary fat. Diet containing polyunsaturated fatty acid would result in more fat absorption and thus less deposition. Comparatively, diet with higher saturated fatty acid profile like crude palm oil or tallow, would result in less fat absorption and thus more abdominal fat deposition (Wiseman 1984). Abdominal fat percentage was significantly reduced (P<0.05) with emulsifier in starter diet, and significantly reduced (P<0.05) with emulsifier and lower ME level in grower diet. Emulsification of triglycerides in micelles formation enhances the activity of pancreatic lipase, thus provides better digestibility and absorption of monoglycerides and fatty acids in the digestive gut (Sanz *et al*. 1999). In modern commercial chicken, inclusion of emulsifier in poultry diet may be considered in dietary composition and feeding strategy, in order to regulate the lipid metabolism and reduce carcass fatness.

Donaldson (1985) reported that calorie-protein ratio had no significant effect on feed conversion, liver weight, liver fat or fatty acid synthetase activity. With constant calorie-protein ratio of 139, a decrease in the total fat level in the diet (8.6, 6.3 and 4.1%) had no effect on bird's liver weight, liver fat or body fat. However, emulsifiers were able to reduce this excessive fat deposition in liver compared with those birds fed with control diets. Fat content in breast meat was increased with age, with significant (P<0.05) positive effect at grower phase. Emulsifiers facilitate fat utilization and absorption into the body by reducing liver fat deposition and subsequently increased (P<0.05) muscle fat content. Fatty acids are synthesized in liver and are transported as triglycerides to the adipose tissue for storage (Hermier 1997). The finding was supported by the lower (P<0.05) abdominal fat deposition in birds that consumed emulsified diets, which resulted in lower (P<0.05) concentration of fat in voided excreta. The decreased fecal fat was supported the finding by Roy *et al*. (2010), who suggested that glyceryl polyethylene glycol ricinoleate can be used as an effective emulsifier for broiler chicken, as an increase of approximate 50% fat accretion was reported in birds that consumed emulsified diet over the control diet.

Apparent digestibility: Fat digestibility was higher in low energy diets (Table 5). Improved energy efficiency was observed with reduced level of dietary energy. The efficiency of fat utilization was found to be higher (P<0.05) in treatments T7, T8 and T9 compared to treatments T4, T5 and T6 with their respective ME levels. A decrease of AME was observed in diets with low ME levels regardless of the types of emulsifier. However, compensation effect by emulsifier on AME recovery back to the basal diet was not observed in any treatment at any level of ME, as ME value of the diet was not returned to the control value. No significant (P>0.05) emulsifier and ME level interaction was observed in apparent metabolizable energy throughout the whole feeding cycle.

Digestive system maturation increases with age and thus increases the AME of feed (Wiseman *et al*. 1998). Fat utilization and the ability to digest and absorb lipids are higher in older birds. Tancharoenrat *et al*. (2010) reported that AME was low during the first week of age, improved in second week, became non-significant improvement in third week and then there was no further improvement after the third week of age. Positive correlation between emulsifier on AME and fat digestibility indicates an efficacy of spherical micelle formation, leading to better fat emulsification in the gastrointestinal tract. Lysophospholipids have higher ability to form smaller micelles which are more effective than bile and soy lecithin (Melegy *et al*. 2010). Due to its molecular structure with one fatty acid residue per molecule, lysophospholipids are more hydrophilic than phospholipids and are capable to form spherical micelles in aqueous solutions (Vasanthakumari *et al*. 2011). Guerreiro *et al*. (2011) reported that an increase of AME was observed in starter and grower phase when birds were fed with emulsified diet with poultry fat as energy source. Significant improvement in ME was observed in young birds especially fed with diets containing saturated oils instead of unsaturated oils (Walker 2011).

It is concluded that supplementation of emulsifier in feed formulation was able to enhance the utilization efficiency in dietary fat and improve bird performance in starter phase. The correlation analysis of the liver fat, abdominal fat and digesta fat provided valuable information on the response effect of emulsifier to the dietary fat, by enhancing nutrient availability to maximize broiler performance. However, this effect of feed efficiency and energy utilization was not significantly transformed into improvement of performance after 14 days of age. The effect of emulsifier in low ME diet was not found in this specific experimental design. Future research is recommended to identify the minimum ME level that can be reduced in formulation besides increasing the inclusion rate of emulsifier.

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