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Effect of Exogenous Emulsifier on Growth Performance, Fat Digestibility, Apparent Metabolisable Energy in Broiler Chickens

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ABSTRACT

This research was done to evaluate the effect of a commercial exogenous emulsifier (polyethylene glycol ricinoleate (PEGR)) with high hydrophilic-to-lipophilic balance (HLB) supplementation to broiler chicken diets on growth performance, digestibility of fat and apparent metabolisable energy (AME) content in week 1, 3 and 5. A total of 360 one-day-old male Cobb broiler chicks were assigned in groups of 30 to 12 battery cages. The chicks were randomly assigned to two dietary treatments, with 6 replicate cages per treatment. The diets were either standard broiler starter and finisher, with rice bran oil (RBO) as supplemented fat source or similar diets + 0.05% emulsifier (RBOV). Feed intakes of RBOV groups significantly increased compared to those of RBO groups from week 2 till 4 while body weights of RBOV diets significantly increased in week 4 and 5. Both RBOV and RBO groups had similar FCR except for week 5. Addition of this strongly hydrophilic emulsifier showed no significant difference in fat digestibility of both RC and RV groups but higher AME was noted for the treatment than for the control groups at week 5. Therefore, supplementing the exogenous emulsifier into a broiler diet enriched with rice bran oil improved body weight and AME content at week 5 with minimal effect on fat digestibility.

INTRODUCTION

Fat is recognised as a high calorie dense ingredient in poultry nutrition. It is known that the ability to utilise fat increases with age [1]. In young birds, the digestion and absorption of dietary fats are limited mainly because of insufficient secretion of bile salts rather than lipase [2]. Hence, they have a low capacity to produce and recirculate bile salts until their digestive system matures (10–14 days of age) [3]. Moreover, production of pancreatic lipase is also stimulated by the release of bile salts that is affected principally by the action of the cholecystokinin (CCK). CCK release is mediated by sensors located in the crypts of the intestinal mucosa, sensitive to the presence of fat.

Therefore, the most limiting process in fat digestibility is not the hydrolysis of triglycerides but the micelle formation [4]. For this reason, bile acid derivatives such as bile salts and cholic acid have been incorporated in diets for young birds to improve fat digestion and absorption [5].

Gomez and Polin [6] supplemented bile acid (cholic and chenodeoxycholic acids) and bile salts (taurocholate) at three different levels namely 0, 0.25 and 0.5 g/kg to a corn-soy based diet and concluded that addition of 0.5 g/kg cholic acid improved the absorption of fat. Supplementation of ox-bile at 5 g/kg also showed to increase weight gain and improve FCR [7]. Although dietary supplementation of bile salts improved fat utilization in chicks, it was not economical [8]. For this reason, emulsifiers can be supplemented to broilers' diet. An emulsifier, by definition, is a surface-active substance that acts on the surface between two media, such as water and fat, which are immiscible. It may mimic and fortify the effect of the natural bile salts in poultry, and thus improving the absorption and utilization of fat in broilers. The emulsification process also depends on the nature of fat, such as the chain length and saturation level [9].

Therefore, the present research evaluated the effect of a commercial hydrophilic emulsifier, Volamel Extra, based on glycerol polyethylene glycol ricinoleate or PEGR (Nukamel N.V., Hoogbuul, Olen, Belgium) on broiler growth performance, digestibility of fat and apparent metabolisable energy (AME) content in week 1, 3 and 5.

MATERIALS AND METHODS

Emulsifier and rice bran oil

The exogenous emulsifier, Volamel Extra was sourced from Nukamel N.V. (Olen, Belgium). The emulsifier was based on 20% polyethylene glycol riconoleate (PEGR) on a carrier of soy, wheat and whey proteins and has a hydrophilic-to-lipophilic balance (HLB value) > 18. This characterizes the hydrophilic nature of the product.

Birds, diets and management

The experiment was carried out following the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia. A total of 360 one-day-old male Cobb broiler chicks were assigned in groups of 30 to 12 battery cages with wire floors in environmentally-controlled rooms. On day 1, temperature was set at 32°C and gradually reduced to 23°C by day 21. All birds were provided feed and water ad libitum throughout the experimental period. The birds were fed standard broiler corn-soybean meal based starter and grower diets (mash form) formulated to meet or exceed Cobb 500 nutritional requirements [10,11] from day 1 to 20 and day 21 to 35, respectively.

The chicks were randomly assigned to one of the two dietary treatments, with 6 replicate cages per treatment. The dietary treatments were basal diet containing rice bran oil without emulsifier supplementation (RBO) and basal diet containing rice bran oil with 0.05% emulsifier supplementation (RBOV). All the diets (**Table** 1) were isocaloric and isonitrogenous. Neither antibiotic growth promoter nor enzyme was added in the experimental diets. Titanium dioxide (TiO_2) was added into the experimental diets as digestibility marker.

Body weight and feed intake were recorded weekly and feed conversion ratios (FCR) were calculated. As for the fat digestibility and AME determination, ileal content was collected during week 1, 3 and 5 posthatch. A total of 15, 5 and 5 chicks respectively from each cages were slaughtered and ileal (from Meckel's diverticulum to the ileo-cecal junction) contents were collected by gentle flushing with distilled water. The digesta samples were oven-dried, finely ground and then kept in -20°C until further analysis.

Table 1. ingredients and nutrients composition of starter and grower diets (%, as-fed basis).

Ingredients	Starter RBO	Starter RBOV	Grower RBO	Grower RBOV
Corn	57.71	57.71	63.27	63.27
Soyabean	35.90	35.90	28.57	28.57
Rice Bran Oil	2.50	2.50	4.50	4.50
Limestone	1.03	1.03	0.93	0.93
Dcp	2.13	2.13	1.93	1.93
Salt	0.33	0.33	0.33	0.33
L-Lysine	0.03	0.03	0.09	0.09
Dl-Methionine	0.12	0.12	0.13	0.13
Choline Chloride	0.10	0.10	0.10	0.10
Mineral Premix[1]	0.10	0.10	0.10	0.10
Vitamin Premix[2]	0.05	0.05	0.05	0.05
Titanium Oxide	0.50	0.50	0.50	0.50
Volamel Extra	-	0.05	-	0.05
			Calculated Composition (%)	
Metabolisable Energy, Kcal/Kg	3013	3013	3208	3208
Crude Protein	21	21	21	21
Crude Fat	5.39	5.39	7.43	7.43
Lysine	1.20	1.20	1.20	1.20
Methionine	0.46	0.46	0.46	0.46
Calcium	1.00	1.00	1.00	1.00
Available Phosphorus	0.50	0.50	0.50	0.50

[1]Supplied per kilogram of the diet: Mn, 100g; Fe, 100g; Zn, 100g; Cu, 10g; I, 2g, Se, 0.3g; Co, 0.3g.
[2]Supplied per kilogram of the diet: vitamin A, 25MIU; vitamin D3, 10MIU; vitamin E, 130g; vitamin K3, 6g; vitamin B1, 4g; vitamin B2, 20g; vitamin B6, 8g; vitamin B12, 0.05g; biotin, 0.25g; folic acid, 4g; niacin, 100g; panthothenic acid, 28g.

Chemical analysis

Determination of TiO_2 concentrations in feed and digesta samples were done according to the procedures of Short *et al.* [12]. Feed and digesta samples were dried in oven at 105°C for 24h to determine dry matter (DM) following the AOAC [13] procedures. Crude fat of feed and digesta samples were determined using ether extract (EE) method according to AOAC [13]. Gross energy was analysed by using PARR oxygen bomb calorimeter.

Fatty acid analysis

Total fatty acids were extracted from rice bran oil by using chloroform : methanol 2 : 1 (v/v) containing butylated hydroxytoluene acoording to the method of Folch *et al.* [14] modified by Rajion *et al.* [15] as described by Ebrahimi *et al.* [16]. The extracted fatty acids were transmethylated to their fatty acid methyl esters (FAME) using 0.66N potassium hydroxide (KOH) in methanol and 14% methanolic boron trifluoride (BF_3) (Sigma Chemical Co., St. Louis, MO, USA) according to the methods by AOAC [13].

The FAME was separated and quantified by gas liquid chromatography on an Agilent 7890A GC model (Agilent, Palo Alto, CA, USA) using a 100 m × 0.25 mm ID Supelco SP-2560 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microliter of FAME was injected by an autosampler into the chromatograph, equipped with a flame ionization detector (FID). Helium (He) gas was used as the carrier gas, and the split ratio was 10 : 1 after injection of the FAME. The injector temperature was programmed at 250°C, and the detector temperature was 300°C. The column temperature program initiated runs at 120°C held for 5 min, increased up to 170°C by 2°C/min, held for 15 min, and then increased up to 200°C by 5°C/min, held at 200°C for 5 min, then increased again to a final temperature of 235°C by 2°C/min, and held for 10 min until the end of the analytical run.

Recoveries and correction factors to determine the individual fatty acid composition were made from a reference standard (mix C4–C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, MO, USA) and CLA standard mix (CLA cis-9 trans-11 and CLA trans-10, cis-12, Sigma-Aldrich, Inc., St. Louis, MO, USA).

Calculations and statistical analysis

The coefficients of fat digestibility and AME contents were calculated using the following formula [17]:

Fat digestibility (%) = 100 – ((TiO_2diet / TiO_2digesta) x (EEdigesta / EEdiet) x 100.

AME (Kcal/kg) = GE diet – (GEdigesta x (TiO_2diet / TiO_2digesta))

All data were subjected to ANOVA using the GLM procedure of SAS [18]. The significant differences among treatments were tested by Duncan's multiple-range test. A level of $P \leq 0.05$ is the criteria for statistical significance

RESULTS AND DISCUSSION

Rice bran oil is highly unsaturated and contains high lipid U/S ratio. The fatty acid composition of rice bran oil is presented in **Table** 2.

Table 2. fatty acid composition of rice bran oil (rbo) (% of total fatty acids methyl esters).

FATTY ACIDS		RBO
C14:0	Myristic Acid	0.36 ± 0.01
C16:0	Palmitic Acid	19.31 ± 0.02
C16:1	Palmitoleic Acid	0.28 ± 0.15
C18:0	Stearic Acid	2.13 ± 0.01
C18:1	Oleic Acid	42.91 ± 0.04
C18:2	Linoleic Acid	33.68 ± 0.03
C18:3	A-Linolenic Acid	1.35 ± 0.10
Total Saturated Fatty Acid		21.80 ± 0.02
Total Unsaturated Fatty Acid		78.21 ± 0.02
Total Mufa		43.18 ± 0.12
Total Pufa N-3		1.35 ± 0.10
Total Pufa N-6		33.68 ± 0.03
N-6:N-3 Ratio		25.05 ± 1.86
Unsaturated:Saturated		3.59 ± 0.00
Polyunsaturated:Saturated		1.61 ± 0.01

$^{a-b}$ means within rows followed by different superscript letters are significantly different ($p \leqslant 0.05$).

The results of growth performance are presented in **Table** 3. As compared to the RBO group, the RBOV birds consumed significantly more feed from week 2 to 4. Emmert *et al.* [19] also observed a higher feed intake in the diet supplemented with choline or deoiled lecithin. On the contrary, Kaczmarek *et al.* [20] found that feed intake was not affected during the entire feeding period. Emulsifier supplementation had a negligible effect on broiler performance for the first two weeks. These findings concur with those of Kaczmarek et al. [20]. However, the bodyweights of the RBOV birds were significantly greater than their RBO counterparts from week 3 to 5. Both RBO and RBOV birds had similar FCR except for week 5 where the latter were less feed efficient. These results suggested that the effect of dietary emulsifier on growth performance may be dependent on age of the birds. Zhang *et al.* [21] and Roy et al. [22] supplemented broilers with lysophosphatidylecholine and PEGR, respectively as exogenous emulsifiers and noted improved growth performance. On the contrary, working with lecithin as exogenous emulsifier in broilers, Blanch *et al.* [23], and Azman and Ciftci [24] noted otherwise. The negligible effect of PEGR as an exogenous emulsifier on performance of ducks was reported by Zosangpuii *et al.* [25].

Table 3. effect of dietary emulsifier supplementation on performance of broilers from 1 – 5 weeks of age (mean±SEM).

	feed intake (g/bird)		bodyweight (g)		cumulative FCR (feed/gain)	
Age (week)	RBO	RBOV	RBO	RBOV	RBO	RBOV
1	148 ± 0.49	149 ± 1.21	184 ± 2.96	180 ± 1.35	0.82 ± 0.01	0.82 ± 0.01
2	401 ± 1.64^{b}	416 ± 1.80^{a}	451 ± 3.32	442 ± 5.17	1.20 ± 0.01	1.19 ± 0.01
3	669 ± 5.85^{b}	709 ± 8.95^{a}	890 ± 9.22^{b}	934 ± 9.49^{a}	1.37 ± 0.01	1.35 ± 0.01
4	1014 ± 4.99^{b}	1043 ± 7.33^{a}	1519 ± 11.16^{b}	1580 ± 10.26^{a}	1.49 ± 0.01	1.47 ± 0.02
5	1185 ± 19.71	1195 ± 18.60	2201 ± 17.68^{b}	2281 ± 14.29^{a}	1.55 ± 0.01^{b}	1.57 ± 0.01^{A}

$^{a-b}$ means within rows followed by different superscript letters are significantly different ($p \leqslant 0.05$).

It appears that discrepancies in results obtained could be attributed to the type of exogenous emulsifier used. Each type of exogenous emulsifier may have different influence on intestinal digestion [26]. Dierick and Decuypere [27] suggested that the inconsistent effect of exogenous emulsifiers on growth performance could be due to the degree of saturation of the dietary fat used. Soares and Lopez-Bote [28] compared the emulsifier effect of dietary lecithin on soybean oil and lard and concluded that the most beneficial effect of lecithin was observed in diets enriched with lard which is higher is saturated fats. Jones *et al.* [26] also found that tallow was more digestible when lecithin and lysolecithin as exogenous emulsifiers were supplemented. The authors indicated that emulsifier was more efficient in improving the digestion of saturated as compared to unsaturated fats through enhancement of the formation of micelles. Similarly, Huyghebaert [29] found that PEGR supplementation was more beneficial to diets containing fats and oils with lower unsaturated:saturated ratio and higher fatty acid content. Furthermore, free fatty acid content and non-nutritive fraction may have also affected the effectiveness of exogenous emulsifiers [23]. In the present study, although rice bran oil contained high unsaturated: saturated fatty acid ratio (**Table** 2) and may have less potential for the exogenous emulsifier to work effectively, body weights of RBOV birds were significantly greater than their RBO counterparts from week 3 onwards. Hence, it appears that supplementation with exogenous emulsifier based on PEGR could be beneficial for birds fed diets containing high unsaturated: saturated fatty acid ratio.

Data on digestibility of fat and apparent metabolisable energy (AME) are shown in **Table** 4. The RBOV chickens showed significantly higher AME at week 5 when compared to the RBO group although both groups had similar AME at week 1 and 3. The beneficial effect of exogenous emulsifiers on nutrient digestibility has been documented in poultry [21] and pigs [26, 30]. In the present study, the fat digestibility of RBO and RBOV birds were not significantly different throughout the experimental period. It could be due to the less pronounced effect of emulsifier on the high unsaturated fat source [29]. A number of factors may contribute to the utilization of supplemental fat including the composition of the supplemental fat, the presence of emulsifiers, and the physical form of the fat [27]. The level of dietary fat may also influence the effectiveness of exogenous emulsifier in aiding digestion [25].

Table 4. effect of dietary emulsifier supplementation on ame and fat digestibility of broilers at 1, 3 and 5 weeks of age (mean±SEM).

	AME (kcal/kg)		fat digestibility (%)	
age (week)	RBO	RBOV	RBO	RBOV
1	2948 ± 67.33	3066 ± 97.42	72.01 ± 1.55	79.14 ± 1.77
3	3476 ± 21.99	3373 ± 31.82	82.58 ± 2.39	84.77 ± 1.37
5	3738 ± 5.48^{b}	3844 ± 7.60^{a}	86.10 ± 0.79	88.66 ± 0.77

$^{a-b}$ means within rows followed by different superscript letters are significantly different ($p \leq 0.05$).

Although fat digestibility was not affected in the present study, at week 5 of age, the RBOV birds had higher AME than those of RBO. Our results confirmed that previous reports of Dierick and Decuypere [27] who observed improvement neither on the apparent ileal and faecal digestibility of fat, but on energy. The improved AME could be attributed to the improved proteolysis effect of emulsifier [31]. The hypothesis was supported by Jones *et al.* [26] who noted increased nitrogen digestibility by feeding lysolecithin in weaning pigs. Despite showing better AME, the FCR of RBOV birds at week 5 were poorer than those of RBO but higher body weight were noted on the RBOV group. Jones *et al.* [26] reported that addition of emulsifiers increased digestibility of nutrients but had minimal effect on growth performance in pigs. Zaefarian *et al.* [32] suggested that the effectiveness of emulsifier was probably within a short period and thus the increased AME may not be sufficient to affect broilers performance.

In conclusion, dietary supplementation of exogenous emulsifier into broiler diet enriched with rice bran oil can improve apparent metabolisable energy and body weight at week 5. The lack of improvement in fat digestion in the present study may be associated with the type of fat used.

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