Korean Journal Of Agricultural Science

pISSN : 2466-2402
eISSN : 2466-2410

ANIMAL

Effects of supplemental glycerol polyethylene glycol ricinoleate in different energy density diets on the growth performance, blood profiles, nutrient utilization, and excreta gas emission of broilers: focus on dietary glycerol polyethylene glycol ricinoleate in broilers

Jia Yin, Hyeok Min Yun, In Ho Kim*

Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea

*Corresponding author: inhokim@dankook.ac.kr

Abstract

This study evaluated the effects of glycerol polyethylene glycol ricinoleate (GPGR) supplementation in different energy density diets on the growth performance, blood profiles, excreta gas emission, and total tract apparent retention (TTAR) of nutrients in broilers. A total of 544 one-day-old male Ross broilers were used in a 35-day trial. The broilers were allocated into one of four treatment groups in a 2 × 2 factorial arrangement with two levels of energy densities (a normal energy or decreased energy density) and GPGR (0 and 0.035%). From day 18 to 35, the GPGR supplemented and normal energy density diet groups showed a significantly improved ($p < 0.05$) body weight gain (BWG). Meanwhile, the GPGR supplemented diet group had a significantly reduced ($p < 0.05$) feed conversion ratio (FCR) compared to that of the non-supplemented diet group. From day 0 to 35, the GPGR supplemented diet and the normal energy density diet groups had a significantly increased ($p < 0.01$) BWG and a reduced ($p < 0.01$) FCR. Moreover, GPGR supplementation tended to increase ($p < 0.1$) the TTAR of the dry matter (DM) compared with the non-supplemented diets. Likewise, the normal energy density diets had a significantly improved TTAR for the gross energy (GE) ($p < 0.05$) than that of the decreased energy density diets. No interactive effects were observed between the energy density and GPGR supplemented diets. In conclusion, both dietary GPGR supplementation and normal energy density diets had beneficial effects on the growth performance of broiler chickens without any adverse effects on blood profiles and excreta gas emission.

Keywords : glycerol polyethylene glycol ricinoleate, energy density, growth performance, total tract apparent retention, broilers

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Citation: Yin J, Yun HM, Kim IH. 2018. Effects of supplemental glycerol polyethylene glycol ricinoleate in different energy density diets on the growth performance, blood profiles, nutrient utilization, and excreta gas emission of broilers: focus on dietary glycerol polyethylene glycol ricinoleate in broilers. Korean Journal of Agricultural Science. https://doi.org/10.7744/kjoas.20180006

DOI: https://doi.org/10.7744/kjoas.20180006
Editor: Jung Min Heo, Chungnam National University
Received: September 27, 2017
Revised: January 31, 2018
Accepted: February 5, 2018

Introduction

Diets are usually required to supply with high nutrient and energy concentrations in order to meet the nutrient requirements of modern intensively reared birds (Patra et al., 2011). Fats and oils are often included to increase energy density in diets of poultry (Zosangpuii et al., 2015). Nutritional emulsifiers can be used to improve fat digestibility and thus improve the energy efficiency. The current evidence indicates that the use of nutritional emulsifiers represents a useful economic strategy to improve the energy value of lipids for poultry (Ravindran et al., 2016; Tan et al., 2016). This will result in a lower feed cost and contribute to a more economical and sustainable animal production (Ravindran et al., 2016). The use and benefits of emulsifier to enhance the energy yield, particularly from poorer quality fats, is an emerging feed technology.

Emulsifiers can help to increase the concentration of monoglycerides in the intestine, and facilitate the nutrient transport through the membrane, allowing a better nutrient absorption and utilization of energy (Zhang et al., 2011; Tan et al., 2016). Many emulsifiers have been evaluated for performance and nutrient utilization in chicken and pigs (Zosangpuii et al., 2015). On the other hand, some researchers reported that the addition of emulsifiers had no positive effects on birds' growth performance (Soares and Lopez-Bote, 2002; Azman et al., 2004).

Addition of glycerol polyethylene glycol ricinoleate (GPGR) as an emulsifier has been shown to improve utilization of dietary fats fed to chicks and ducks (Roy et al., 2010; Patra et al., 2011). Udomprasert and Rukkwamsuk (2006) found that GPGR may improve average daily weight gain and feed conversion ratio (FCR) of weanling pigs. So, the aim of the trial was to evaluate the effects of GPGR supplementation in a normal and decreased energy density diet, as well as the main and interactive effects of GPGR supplementation and dietary energy density on growth performance and total tract apparent retention (TTAR) of nutrients in broilers.

Materials and Methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University.

Emulsifier

The exogenous emulsifier which was named Excential Energy Plus was supplied by Woogene B&G company (Seoul, South Korea). The emulsifier was based on 50% glycerol polyethylene glycol riconoleate (GPGR) on a mineral carrier and has a hydrophilic-to-lipophilic balance (HLB value) > 16. This characterizes the hydrophilic nature of the product. Diets NED and DED were offered either without supplementation of an emulsifier or supplemented with commercially produced GPGR at concentrations of 0.035%. When added to the experimental diets, GPGR was premixed with water.

Experimental design, animals, and housing

A total of 544 one-day-old Ross 308 broiler chickens (BW, body weight = 42 ± 0.76 g) were used in a 35-

day experiment. Broilers were allotted into one of four treatment groups in a 2 × 2 factorial arrangement with two levels of energy density (normal energy or decreased energy density) and GPGR (0 and 0.035%). The 8 replicated pens of 17 birds were randomly assigned to per treatment. The experiment was conducted in 2 phases consisting of a starter phase (1 to 17 d) and a grower phase (18 to 35 d). The normal diets were formulated to meet or exceed the NRC (1994) requirements for broiler chickens (Table 1). Broiler chickens were housed in the animal building of Dankook University and practiced an all-in and all-out production system. The room was cleaned every week during the experiment and it was sterilized regularly by using a disinfectant. The temperature of the room was maintained at 33℃ for the first 3 d. Then gradually decreased by 3℃ per week to 24℃ until the end of the experiment. The humidity was kept around 60% through the whole experiment. Artificial light was provided 24 h per d by fluorescent lights.

Table 1. Compositions of basal broiler chicken diets (%, as-fed basis).

Item	Starter (days 0 - 17)		Grower (days 18 - 35)	
	Normal energy	Decreased energy	Normal energy	Decreased energy
Ingredients (%)				
Wheat	36.27	38.42	40.92	43.43
Soybean meal	34.86	34.36	28.86	28.15
Maize	20.00	20.00	21.00	21.00
Soybean oil	2.89	2.06	3.23	2.33
Lard	2.89	2.06	3.23	2.33
Monocalcium phosphate	0.68	0.68	0.59	0.59
Limestone	0.33	0.33	0.27	0.26
NaCl	0.17	0.17	0.17	0.17
$NaHCO_3$	0.25	0.25	0.20	0.20
Methionine	0.28	0.28	0.21	0.21
Lysine-HCl	0.28	0.29	0.26	0.27
Threonine	0.10	0.10	0.06	0.06
Vitamin premix[y]	0.55	0.55	0.55	0.55
Mineral premix[z]	0.45	0.45	0.45	0.45
Total	100.00	100.00	100.00	100.00
Calculated value (%)				
Metabolizable energy (kcal/kg)	3030	2930	3100	3000
Crude Protein	23.60	23.50	19.90	19.80
Ca	0.90	0.89	0.83	0.84
Available Phosphorus	0.56	0.56	0.54	0.54
Lysine	1.15	1.15	0.95	0.95
Methionine	0.63	0.63	0.56	0.56
Methionine + cysteine	0.94	0.94	0.76	0.76
Threonine	0.71	0.71	0.61	0.61
Tryptophan	0.22	0.22	0.19	0.19

[y]Provided per kilogram of diet: vitamin A, 11,250 IU; vitamin D_3, 2,500 IU; vitamin E, 80 mg; vitamin B_{12}, 0.02 mg; niacin, 41.67 mg; folic acid, 1.17 mg; biotin, 0.18 mg; pantothenic acid, 12.5 mg; menadione, 2.50 mg; choline, 379 mg; riboflavin, 7.0 mg; thiamin, 2.17 mg; pyridoxine, 4.0 mg; ethoxyquin, 0.09 mg.

[z]Provided per kilogram of diet: Cu, 20 mg; Zn, 55 mg; Mn, 73 mg; Fe, 45 mg; Se, 0.3 mg; salinomycin, 60mg.

Experimental procedures and sampling

The broiler chickens were weighed by cage, and body weight was recorded on days 1, 7, 17 and 35 to calculate body weight gain (BWG), feed intake (FI) and FCR for each feeding phase. Chromic oxide (0.2%) was added to diets for 7 days before excreta collection to determined TTAR of dry matter (DM), gross energy (GE), and nitrogen (N). At the end of the experiment, 24 broilers were randomly selected from each treatment (3 birds per cage). Blood samples were collected from the wing vein into a sterile syringe and stored at -4℃. Samples for serum analysis were then centrifuged at 3000 × g for 15 min and serum was separated. The concentrations of triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in the serum samples were analyzed with an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY, USA) using colorimetric methods.

The fresh excreta samples were collected from each pen on day 35. The samples were stored in a freezer at -20℃ until analyzed. Subsamples of excreta were taken and stored in 2.6 L plastic boxes in duplicate, and plastic boxes were sealed carefully. Each box had a small hole in the middle of one side wall, which was sealed with adhesive plaster. The samples were permitted to ferment for 5 d at room temperature (25℃). After fermentation period, a gas sampling pump (Model GV-100; GASTEC Corp. Ayase, Kanagawa, Japan) was utilized for gas detection. Concentrations of NH3, H2S, and R.SH were measured by using a detector tube (GASTEC Corp.) within scope of 5.0 - 100.0 (No. 3La, detector tube; GASTEC Corp.), 2.0 - 20.0 (No. 4LK, detector tube; GASTEC Corp.), and 0.5 - 120.0 (No. 70L, detector tube; GASTEC Corp.). Before measurements, slurry samples were shaken manually for approximately 30 s to disrupt any crust formation on the surface of the slurry sample and to homogenize samples. The adhesive plasters were punctured, and 100 mL of headspace air was sampled approximately 2.0 cm above the slurry surface. Two samples from each cage were measured and then the average was calculated.

Chemical analysis

Before chemical analysis, feed and digest samples were thawed and dried at 70℃ for 72 h. Then they were finely ground to a size that could pass through a 1-mm screen. Chromium was analyzed by UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). GE was determined by measuring the heat of combustion in the samples using a bomb calorimeter (Parr 6100, Parr instrument Co., Moline, IL, USA). Dietary DM (method 930.15), CP (method 968.06), calcium (method 984.01), and phosphorus (method 965.17) were analyzed according to the procedures described by AOAC (AOAC, 2005). N was determined by a Kjectec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden).

Calculations and statistical analysis

TTAR was calculated as follows: TTAR = 1 - $[(C_i \times N_o) / (C_o \times N_i)]$, where: C_i = the concentration of chromium in the diet, C_o = the concentration of chromium in the excreta, N_o = the concentration of the nutrient in excreta, and N_i = the concentration of the nutrient in the feed (Olukosi et al., 2008).

All data were analyzed as a completely randomized block design with 2 × 2 factorial arrangement using generalized linear model procedures of the SAS software (SAS Institue, 2001). The main effect included dietary energy densities (normal vs. decreased) and GPGR addition. For all response criteria, the pen served as the experimental unit. Variability of data was expressed as standard error of means, and $p < 0.05$ was considered statistically significant whereas $p < 0.10$ was considered a trend.

Results and Discussion

Growth performance and blood profiles

Data on BWG, FI, FCR during experimental stage of broiler chickens fed with GPGR and different energy densities were presented in Table 2. Form days 0 to 17, no difference ($p > 0.05$) was observed in response to GPGR supplementation and energy density. From days 18 to 35, broilers fed the GPGR supplemented diet had a higher ($p < 0.01$) BWG, a lower ($p < 0.05$) FCR and tended to increase FI ($p < 0.10$) than those broilers fed a diet without GPGR. Meanwhile, broilers fed the normal energy density diet had a higher BWG ($p < 0.05$) and tended to reduce FCR ($p < 0.1$) than those broilers fed the decreased energy density diet. Overall, energy density and the supplementation of GPGR had no effect on FI. Broilers fed the GPGR supplemented diet had a higher ($p < 0.001$) BWG and a lower ($p < 0.001$) FCR than those broilers fed a diet without GPGR. Likewise, broilers fed the normal energy density diet had a

Table 2. Effects of GPRP in different energy density diets on growth performance in broilers.

Items	NED		DED		SEM[z]	p-value		
	Emulsifier -	Emulsifier +	Emulsifier -	Emulsifier +		ED	Emulsifier	ED × Emulsifier
Days 0 - 7								
BWG (g)	128	129	125	130	3.25	0.84	0.37	0.49
FI (g)	165	161	163	162	4.50	0.80	0.54	0.74
FCR	1.292	1.252	1.301	1.239	0.05	0.94	0.33	0.89
Days 8 - 17								
BWG (g)	505	522	496	518	12.71	0.61	0.13	0.85
FI (g)	694	691	692	694	9.27	0.97	0.94	0.78
FCR	1.374	1.322	1.395	1.338	0.03	0.52	0.12	0.87
Days 18 - 35								
BWG (g)	931	990	915	943	12.99	0.02	< 0.01	0.23
FI (g)	1554	1572	1548	1565	9.14	0.51	0.06	0.96
FCR	1.67	1.609	1.692	1.661	0.03	0.08	0.04	0.40
Days 0 - 35								
BWG (g)	1563	1641	1536	1591	11.60	< 0.01	< 0.001	0.34
FI (g)	2413	2424	2402	2421	10.96	0.54	0.20	0.74
FCR	1.544	1.489	1. 565	1.521	0.01	< 0.01	< 0.001	0.28

GPRP, glycerol polyethylene glycol ricinoleate; BWG, body weight gain; FCR, feed conversion ratio; FI, feed intake; NED, normal energy density; DED, decreased energy density; Emulsifier-, diet without addition of 0.035% GPRP; Emulsifier+, diet with addition of 0.035% GPRP; ED, energy density.

[z]Standard error of the mean.

higher ($p < 0.01$) BWG and a lower ($p < 0.01$) FCR. No interactive effect on growth performance was observed among treatments. No difference ($p > 0.05$) was observed in blood profiles (Table 3).

GPGR increased BWG, reduced FCR and tended to increase FI from day 18 to 35. The result is in agreement with Roy et al. (2010), who revealed that GPGR may be considered as a feed additive component in the dietary regimen of high-yielding broiler chickens for augmenting nutrient utilization and food conversion in broilers. However, there was no significant influence of GPGR supplementation on broiler performance from day 0 to 17. Our study is in agreement with Kaczmarek et al. (2015), who revealed that emulsifier supplementation had a negligible effect on broiler performance for the first two weeks. It is well known that emulsifiers can reduce the surface tension of water and increase the penetration and improve the distribution of water in press meal (Van der Heijden and de Haan, 2010). In the current study, the emulsifier product was mixed with feed compounds before pelleting process, which may increase humidity, reduce pellet press energy consumption and improve pellet quality by modulating the moisture content during the pelleting process. Consequently, the emulsifier product had improved feed intake and performance of animals (Bontempo et al., 2016).

Effects of dietary energy density on the performance of broilers have been studied extensively (Rabie et al., 1998; Hidalgo et al., 2004). Corduk et al. (2007) reported that dietary energy levels had a significant effect on BWG and FCR during all feeding broiler periods. Likewise, they indicated that increasing dietary energy resulted in higher BWG, which was consistent with the current results.

TTAR of nutrients and excreta gas emission

The effects of dietary treatments on TTAR of DM, N and GE were presented in Table 4. No interactive effect on the TTAR of DM, N and GE was among treatments. On day 35, broilers fed the GPGR supplemented diet had tended to increase ($p < 0.1$) DM retention than those broilers fed a diet without GPGR, and had no effect on TTAR of GE ($p > 0.05$). Likewise, broilers fed the normal energy density diet had a higher GE retention ($p < 0.05$) than those fed the decreased energy density diet, and had no effect on TTAR of DM ($p > 0.05$). However, GPGR supplementation and energy density did not improve ($p > 0.05$) TTAR of N. No difference ($p > 0.05$) was observed in excreta gas emission (Table 5).

Table 3. Effects of GPRP in different energy density diets on blood profiles in broilers.

Items (mg/dL)	NED		DED		SEM[z]	p-value		
	Emulsifier-	Emulsifier+	Emulsifier-	Emulsifier+		ED	Emulsifier	ED × Emulsifier
Total cholesterol	120	118	118	121	3.56	0.92	0.88	0.47
HDL-cholesterol	87	89	84	86	3.34	0.37	0.53	0.95
LDL-cholesterol	29	26	30	28	2.03	0.37	0.20	0.94
Triglyceride	61	57	64	62	3.31	0.24	0.38	0.73

GPRP, glycerol polyethylene glycol ricinoleate; NED, normal energy density; DED, decreased energy density; Emulsifier-, diet without addition of 0.035% GPRP; Emulsifier+, diet with addition of 0.035% GPRP; ED, energy density; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol.

[z]Standard error of the mean.

Positive effect of emulsifiers on nutrients utilization is documented in broilers (Jansen et al., 2015; Bogusławska-Tryk et al., 2016). In increased energy density diets, GPGR has been proven to increase the nutrient utilization and energy values in feed (Tan et al., 2016). Similarly, supplemental GPGR in the present study increased TTAR of DM. These results agreed with the observations of Roy et al. (2010), who showed that addition of GPGR in broiler diets increased the metabolizability of DM and fat. Lecithin, an emulsifier, has been reported to depress free fatty acid absorption, probably by increasing the size of bile salt micelles (Saunders and Sillery, 1976). According to Dierick and Decuypere (2004) research, compared to lecithin, GPGR is more hydrophilic and dissolves the free fatty acids, which are largely insoluble in bile salt micelles alone and thereby increases the digestibility of saturated fatty acids. Moreover, they reported that the addition of GPGR improved the digestibility of major nutrients, which may reduce the viscosity of the digestive contents and increase the transit of the digest as well as feed intake. The use of GPGR could solubilize fats, and hence improve the absorption of the fatty acids from the gut.

GE was significantly affected by calculated energy density (Moritz et al., 2003). The normal energy density diet yielded higher TTAR of GE in the present study. Similarly, Meng et al. (2010) reported that pigs fed the high-energy diets had greater energy digestibility ($p < 0.01$) compared with those fed the low-energy diets. In turn, the higher TTAR of nutrients in treatments may explain the increased growth performance in the present study. High energy densities are linked to high performance while reduction in dietary energy is not always associated with negative effects (Tang et al., 2007). The effects were associated with feed intake, indicating that broilers increased energy intake when a relatively high nutrient density diet was provided.

Table 4. Effects of GPRP in different energy density diets on TTAR in broilers.

Items (%)	NED		DED		SEM[z]	p-value		
	Emulsifier-	Emulsifier+	Emulsifier-	Emulsifier+		ED	Emulsifier	ED × Emulsifier
Day 35								
Dry matter	72.7	74.9	72.5	73.1	0.69	0.15	0.05	0.26
Nitrogen	69.7	71.2	68.3	69.8	0.98	0.16	0.14	0.99
Gross energy	74.2	75.2	72.7	73.3	0.75	0.03	0.29	0.82

GPRP, glycerol polyethylene glycol ricinoleate; TTAR, total tract apparent retention; NED, normal energy density; DED, decreased energy density; Emulsifier-, diet without addition of 0.035% GPRP; Emulsifier+, diet with addition of 0.035% GPRP; ED, energy density.

[z]Standard error of the mean.

Table 5. Effects of GPRP in different energy density diets on excreta gas emission in broilers.

Items (ppm)	NED		DED		SEM[z]	p-value		
	Emulsifier-	Emulsifier+	Emulsifier-	Emulsifier+		ED	Emulsifier	ED × Emulsifier
NH_3	29.0	28.2	30.2	29.6	0.67	0.06	0.28	0.89
H_2S	2.36	2.27	2.49	2.32	0.20	0.65	0.51	0.84
R.SH	1.32	1.21	1.36	1.45	0.13	0.27	0.94	0.44

GPRP, glycerol polyethylene glycol ricinoleate; NED, normal energy density; DED, decreased energy density; Emulsifier-, diet without addition of 0.035% GPRP; Emulsifier+, diet with addition of 0.035% GPRP; ED, energy density.

[z]Standard error of the mean.

The effects of dietary exogenous emulsifier supplementation have been shown to be affected by intrinsic and extrinsic factors, including the environment, diet and nutritional status (Tancharoenrat et al., 2013; Wang et al., 2016). Zaefarian et al. (2015) suggested that GPGR may specifically enhance the activities of digestive enzymes and fat absorption, which may improve the biological values of increased energy density diets. In the current study, no interactive effects were observed between GPGR supplementation and dietary energy density on growth performance and TTAR of nutrients. The exact reason for this is unknown; therefore, further studies should be conducted to evaluate this interactive effect.

Conclusion

This study suggests that both dietary GPGR supplementation and normal energy density diets can improve growth performance in broilers. Overall, GPGR is considered as a feed additive component in the dietary broilers for augmenting nutrient utilization and food conversion in broilers.

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