Short Communication

Effects of supplementing varying levels of 1,3-diacylglycerol (DAG) on growth performance, apparent nutrient digestibility and selected fecal microbial populations in growing pigs

Wen Chao Liu[1#] Kwan Sik Yun[2#] In Ho Kim[3*]

Abstract

The objective of the present study was to investigate the effects of 1,3-diacylglycerol (DAG) supplementation on growth performance, apparent digestibility and selected fecal microbial populations in growing pigs. A total of 80 crossbred pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight (BW) of 27.52 ± 0.20 kg were used in a 6 week feeding trial. Pigs were allotted to 4 dietary treatments based on their initial BW and sex (5 replications; 4 pigs per pen with 2 barrows and 2 gilts). Dietary treatments included: 1) CON, basal diets; 2) CON+0.075% 1,3-DAG; 3) CON+0.10% 1,3-DAG; 4) CON+0.15% 1,3-DAG. The final BW was linearly increased (P=0.0056) by 1,3-DAG supplementation. Average daily feed intake (ADFI) was improved (cubic, P=0.0312) by feeding 1,3-DAG during week 1-3. Overall (week 1-6), dietary 1,3-DAG supplementation led to a linearly increase in ADG (P=0.0042) and G:F (P=0.0235). In addition, dietary supplementation of 1,3-DAG linearly improved (P=0.0004) the apparent digestibility of gross energy and had a tendency (linear, P=0.0725) to improve the apparent digestibility of crude fat. Pigs fed 1,3-DAG supplemented diet also linearly decreased (P=0.0065) fecal *E. coil* populations at week 3 and linearly increased (P=0.0348) fecal *Lactobacillus* populations at week 6. In conclusion, these results show that 1,3-DAG can be used as a potential functional feed additive and exerts positive effects on growth performance, apparent energy digestibility and fecal microbiota in growing pigs.

Keywords: 1,3-diacylglycerol, growing pigs, growth promoter

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Introduction

There is general consensus that energy intake and utilization are often the first limiting factors for protein deposition in young and growing pigs (Nieto et al., 2002), thus greatly affecting productive performance. Dietary energy is mainly produced from the oxidation of lipids, carbohydrates and proteins. Energy generated from the oxidation of fatty acids derived from dietary lipids is more than twofold higher than that generated from starch (van Milgen et al., 2001). Therefore, dietary lipids can be an important source of energy in pig diets. However, there are some problems with lipids digestion and utilization in young and growing pigs due to the immature physiological functions and a low level of natural lipase production (Doreau and Chilliard, 1997).

Diacylglycerol (DAG), a glycerol derivative, possesses 2 hydroxyl groups substituted by fatty acids through ester bond formation and exists either as 1,2-DAG and 1,3-DAG (Flickinger and Matsuo, 2003). The most popular function of DAG is related to their amphiphilic nature and surface-active properties, being well-known as emulsifier ingredients in the food industry (Martin et al., 2014). In this regards, DAG has been used synergistically with monoacylglycerol as an emulsifier to improve the fat digestibility (Flickinger and Matsuo, 2003; Shimada and Ohashi, 2003; Nakajima, 2004). On the other hand, among the two kinds of DAG, 1,3-DAG are hydrolyzed to free fatty acids, 1(3)-monoglycerides (MG) during digestion. Several bioactivities of 1(3)-MG have been reported, including antiviral and antimicrobial (Lieberman et al., 2006), antioxidant (Cho et al., 2010), and the stimulation of fat utilization (Shimotoyodome et al., 2012). Furthermore, DAG can be absorbed directly into the blood and enter hepatocytes via the portal vein for rapid oxidation (Murata et al., 1997). Previous studies have demonstrated that DAG, particularly 1,3-DAG has the ability to suppress body fat accumulation, reduce postprandial hyperlipidemia and serum triacylglycerides (Flickinger and Matsuo, 2003; Yanai et al., 2007), suggesting that 1,3-DAG is involved in lipid metabolism. More recently, our group has shown that dietary supplementation of 1,3-DAG could be used as an emulsifier and induce a greater fat digestibility and growth performance in broilers (Upadhaya et al., 2017). However, to the best of our knowledge, there is no relevant research to evaluate the effects of 1,3-DAG on growth performance in pigs. We hypothesized that dietary 1,3-DAG can rapidly supply energy for growing pigs, and improve lipids digestion and promote gut healthy status, consequently, benefiting the growth performance. The present study was conducted to test this hypothesis.

Materials and Methods

The Animal Care and Use Committee of Dankook University (Cheonan, South Korea) approved all animal feeding, management and experimental data collection procedures of the present study.

Experiment design, animals and housing: In this 6 weeks feeding experiment, a total of 80 crossbred pigs [(Landrace × Yorkshire) × Duroc] were used. Pigs with an average initial BW of 27.52 ± 0.20 kg, and were sorted by sex and balanced by BW assigned to treatment. There were five replicate pens per treatment with four pigs (2 gilts and 2 barrows) per pen. Dietary treatment groups included: 1) CON, basal diets; 2) CON+0.075% 1,3-DAG; 3) CON+0.10% 1,3-DAG; 4) CON+0.15% 1,3-DAG. The tested product, 1,3-DAG (50%) mixed with a carrier was obtained from a commercial company (Il Shin Wells, Korea). The diet was in mash form and formulated to meet or exceed the NRC (2012) nutrient requirements (Table 1). All the pigs were reared in an environmentally controlled house with a slatted plastic floor. Throughout the trial period, a self-feeder and nipple waterer were equipped in each pen, in order to allow the pigs *ad libitum* access to feed and water.

Sampling and measurements: At the beginning of the trial and at the end of weeks 3 and 6, the BW of each pig was measured and the average daily gain (ADG) was calculated. Meanwhile, the feed intake was recorded based on pens throughout the trial period to calculate average daily feed intake (ADFI) and gain: feed (G:F). During the trial, the dead pigs in each pen were also recorded to correct the feed intake.

At the end of week 5, pigs were fed a diet mixed with chromium oxide (Cr_2O_3, 0.2%) as an indigestible marker to calculate the apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen (N), cross energy (GE) and crude fat (Ball and Aherne, 1987). On the last two days of the experiment, fresh fecal samples from each pig were directly collected by massaging, and then they were uniformly pooled based on pen. All fecal samples and feed samples were stored in a -20 ° C freezer until analysis. Fecal samples were dried at 57 °C for 72 hours prior, after which they were ground to pass through a 1-mm sieve. The feed samples were ground to pass through a 1 mm sieve. Then according to (Method 930.15, AOAC 1995) and (Method 990.03, AOAC 1995) the DM and N content were analyzed. The fat content was determined by standard procedures (Method 954.02; AOAC, 2005). The chromium content was determined by UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The energy was determined using a Compensated Jacket Calorimeter 6100 (Parr Instrument Co., Moline, IL USA). Then the following formula was used to calculate the ATTD:

$$ATTD\ (\%) = [1-\{(Nf \times Cd)/(Nd \times Cf)\}] \times 100$$

where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

At the end of week 3 and week 6, for determining the fecal microbiota, fresh fecal samples were collected into sterilized EP tubes from two pigs (one gilt and one barrow) in each pen. Fecal samples were placed on ice and immediately transferred to the laboratory for microbial colony analysis. Fecal samples (1 g) from each pig were diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenized. The fecal samples were then diluted 10-fold (ranging from 10-1 to 10-8) and subsequently cultured on Rogosa SL agar (Rogosa;

Difco Laboratories, Detroit, MI, USA) for counting *Lactobacillus* counts and MacConkey agar (Difco Laboratories, Detroit, MI) was used to calculate *E. coli* counts. All the dishes were inverted and anaerobically cultured at 37 °C for 48 hours after inoculation. After culture, colony counts were calculated and the results expressed as log10 transformed data.

Table 1 Compositions of the basal diets (as-fed basis)

Items	
Ingredient, g/kg	
Corn	600
Soybean Meal 33% CP	161.9
Wheat	60
Distiller's dried grains with solubles	65
Rapeseed meal	25
Molasses	30
Tallow	30
Dicalcium phosphate	11
Limestone	8
L-Lys HCl	2.7
Vitamin premix[1]	2
Mineral premix[2]	1
Salt	3
Choline, 25%	0.4
Calculated composition, g/kg	
Metabolizable energy, kcal/kg	3,302
Crude protein	155
Crude fat	60.3
Calcium	6.5
Total phosphorus	5.5
Avilable phosphorus	3.1
SID Lys	7.2
SID Met	1.8
SID Met+Cys	6.1
Analyzed composition, g/kg	
Gross energy, kcal/kg	3863
Crude protein	157
Crude fat	61
Calcium	7.4
Phosphorus	5.2

[1] Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 µg vitamin B_{12}.

[2] Provided per kg of complete diet: 12 mg Cu (as $CuSO_4 \bullet 5H_2O$); 85 mg Zn (as $ZnSO_4$); 8 mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15 mg Se (as $Na_2SeO_3 \bullet 5H_2O$).

Statistical analysis: All experimental data was analyzed as a randomized complete design using MIXED procedure of SAS 2003 (v. 9.1, SAS Institute Inc., Cary, NC). The linear, quadratic and cubic polynomial contrasts were used to determine responses to supplemental graded levels of 1,3-DAG at

0, 0.075%, 0.10% and 0.15%. Tukey's range test was used for detecting the differences among treatment groups. Variability of the data was expressed as the standard error of means (SEM). Differences were deemed significant when P<0.05. Trends were noted when 0.05 <P<0.10.

Results and Discussion

Growth performance: As described in Table 2, at the end of the experiment, dietary supplementation of 1,3-DAG linearly increased the BW (P=0.0056). During week 1 to week 3, dietary 1,3-DAG supplementation led to an increase in ADFI (cubic, P=0.0312). During week 1 to week 6, dietary supplementation with 1,3-DAG had a linear effect on improving the ADG (P=0.0042) and G:F (P=0.0235). Similar to our results, Upadhaya et al. (2017) showed that supplementation of graded levels of 1,3-DAG linearly improved the weight gain and feed conversion ratios. However, to our knowledge, this is the first report on the effects of 1,3-DAG on the performance of pigs, thus no more comparisons can be made with other studies. The DAG possesses surface-active properties and is well known as an emulsifier ingredient in the food, pharmaceutical and cosmetic industries (Martin et al., 2014). It has been suggested that dietary emulsifier could improve growth performance in weaning pigs (Zhao et al., 2015), finishing pigs (Zhao et al., 2016), lactating sows (Zhao et al., 2016), and broilers (San Tan et al., 2016). Therefore, the positive effects on growth performance of growing pigs may be attributed in part to the emulsifier properties of 1,3-DAG. On the other hand, 1,3-DAG could be absorbed directly into the blood and rapidly supply energy for the body (Murata et al., 1997). Furthermore, the hydrolyzate from 1,3-DAG exerts bactericidal and virucidal effects (Lieberman et al., 2006), consequently promoting gut health status and growth performance. The exact mechanism behind the effects of 1,3-DAG on pig performance, however, remain to be elucidated.

Table 2 Effects of dietary 1,3-diacylglycerol (DAG) supplementation on growth performance in growing pigs[1]

Items[2]	CON	DAG0.075	DAG0.10	DAG0.15	SEM[3]	p-value		
						Linear	Quadratic	Cubic
BW (kg)								
Initial	27.5	27.5	27.5	27.6	0.2	0.3153	0.5628	0.6036
Week 3	41.3	41.5	41.7	41.8	0.4	0.3602	0.9668	0.9554
Week 6	57.1[a]	57.8[ab]	58.3[b]	58.4[b]	0.2	0.0056	0.2145	0.8122
Week 1-3								
ADG (g)	655.1	666.3	671.6	678.0	16.3	0.3144	0.8626	0.9745
ADFI (g)	1527.4	1503.2	1544.7	1502.1	12.0	0.5590	0.4648	0.0312
G:F	0.429	0.443	0.435	0.452	0.01	0.2734	0.9383	0.3544
Week 4-6								
ADG (g)	754.3	777.5	792.6	789.2	14.1	0.1054	0.3701	0.8611
ADFI (g)	1785.1	1772.0	1784.4	1799.8	44.3	0.7824	0.7644	0.9187
G:F	0.422	0.439	0.445	0.439	0.01	0.3746	0.4150	0.9870
Overall								
ADG (g)	704.2[a]	721.7[ab]	732.9[b]	734.4[b]	5.1	0.0042	0.1370	0.8740
ADFI (g)	1656.0	1637.4	1664.3	1651.0	19.3	0.9060	0.9211	0.3627
G:F	0.425[a]	0.441[ab]	0.440[ab]	0.445[b]	0.004	0.0235	0.2124	0.2831

[a,b] Means in the same row with different superscripts differ (P<0.05).

[1] Dietary treatments were as follows: CON: basal diet (antibiotic-free); DAG0.075: CON+0.075% 1,3-DAG; DAG0.10: CON+0.10% 1,3-DAG; DAG0.15: CON+0.15% 1,3-DAG.

[2] There were five replication pens of 4 pigs/pen per treatment. BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

[3] Standard error of the means.

Apparent nutrient digestibility: ATTD of gross energy was significantly improved (P=0.0004) by dietary 1,3-DAG supplementation. In addition, dietary 1,3-DAG supplementation tended to improve (linear, P=0.0725) the ATTD of crude fat (Table 3). Similarly, Upadhaya et al. (2017) reported that the addition of 1,3-DAG to low energy diet increased the ATTD of gross energy in broilers. Other studies also indicated that exogenous emulsifier could improve apparent metabolisable energy (AME) digestibility in broilers (San Tan et al., 2016) and ATTD of gross energy in weaning pigs (Zhao et al., 2015). Therefore, the improved growth performance observed in our study is associated at least in part with the improvement in energy digestibility elicited by 1,3-DAG. Nevertheless, dietary supplementation of 1,3-DAG showed only a slight effect in improving ATTD of crude fat (P=0.0725) in this study. These findings are partially in agreement with an earlier report showing that a diet with exogenous emulsifier had no effect on fat digestibility

but improved AME digestibility in broilers (San Tan et al., 2016). However, the results from the present study are not consistent with previous findings in that 1,3-DAG or emulsifier significantly increased the ATTD of fat in broilers and weaning pigs (Zhao et al., 2015; Upadhaya et al., 2017; Zhao and Kim, 2017). This is probably due to the inclusion level or the age of animals. For instance, Zhao and Kim (2017) suggested that dietary emulsifier improved crude fat digestibility in broilers at d 14 of the age, whereas had no significant effects on crude fat digestibility at d 28 of the age. However, the data of ATTD is only from the end of trial in our study, therefore, it is necessary to cover more stages in future research to study whether 1,3-DAG as a fat emulsifier can improve the digestibility of crude fat. Meanwhile, the data from Zhao and Kim (2017) also indicated that the improvement in crude fat digestibility by emulsifier is more profound at a high inclusion level. Again, it is assumed that the beneficial effects of 1,3-DAG on ATTD could be variable depending on other factors, such as administration strategies and the physical form of the fat in diet.

Table 3 Effects of dietary 1,3-diacylglycerol (DAG) supplementation on apparent nutrient digestibility in growing pigs[1]

Items[2], %	CON	DAG0.075	DAG0.10	DAG0.15	SEM[3]	p-value		
						Linear	Quadratic	Cubic
Dry matter	76.4	77.0	77.2	77.2	0.45	0.1951	0.5737	0.9607
Nitrogen	75.9	76.0	75.9	76.0	0.49	0.8907	0.9707	0.8916
Gross energy	76.4[a]	77.2[ab]	77.9[ab]	78.5[b]	0.38	0.0004	0.8397	0.9837
Crude fat	59.8	61.2	62.3	62.9	1.24	0.0725	0.7572	0.9288

[a,b] Means in the same row with different superscripts differ (P<0.05).

[1] Dietary treatments were as follows: CON: basal diet (antibiotic-free); DAG0.075: CON+0.075% 1,3-DAG; DAG0.10: CON+0.10% 1,3-DAG; DAG0.15: CON+0.15% 1,3-DAG.

[2] There were five replication pens of 4 pigs/pen per treatment.

[3] Standard error of the means.

Selected fecal microbial populations: Results of fecal microbial populations are shown in Table 4, dietary 1,3-DAG supplementation linearly decreased (P=0.0065) fecal *E. coil* populations at the end of week 3, and linearly increased (P=0.0348) fecal *Lactobacillus* populations at the end of week 6. 1,3-DAG is hydrolyzed to 1(3)-monolaurin by lipase *in vivo*. Monolaurin has profound antiviral and antibacterial activity (Lieberman et al., 2006) with a number of different mechanisms proposed by Projan et al. (1994), Zhang et al. (2016) and Zhang and Houtman (2016). Our results showed a linear decline in fecal *E coli* counts at week 3 and a linear increase in *Lactobacillus* counts at week 6 with increasing dietary 1,3-DAG level. This may reflect the antibacterial actions of the hydrolysed products of DAG and certainly warrants further research given the emphasis on antibacterial resistance and reduced antimicrobial use in animal agriculture now.

Table 4 Effects of dietary 1,3-diacylglycerol (DAG) supplementation on selected fecal microbial populations in growing pigs[1]

Items[2], log10cfu/g	CON	DAG0.075	DAG0.10	DAG0.15	SEM[3]	p-value		
						Linear	Quadratic	Cubic
Week 3								
Lactobacillus	7.23	7.29	7.20	7.30	0.11	0.8742	0.9565	0.8660
E. coli	6.40[a]	6.32[ab]	6.24[ab]	6.15[b]	0.06	0.0065	0.8973	0.9302
Week 6								
Lactobacillus	7.03	7.04	7.16	7.14	0.04	0.0348	0.7768	0.2238
E. coli	6.28	6.34	6.36	6.39	0.05	0.7080	0.7532	0.8510

[a,b] Means in the same row with different superscripts differ (P<0.05).

[1] Dietary treatments were as follows: CON: basal diet (antibiotic-free); DAG0.075: CON+0.075% 1,3-DAG; DAG0.10: CON+0.10% 1,3-DAG; DAG0.15: CON+0.15% 1,3-DAG.

[2] There were five replication pens of 4 pigs/pen per treatment.

[3] Standard error of the means.

However, we noted that the selective antibacterial effect of 1,3-DAG on fecal *E. coil* only occurred in week 3 but not in week 6 of the trial, this phenomenon may be attributed to a better developed digestive system, enhanced resistance to intestinal disorders and improved immunity as pigs become older. Regarding the fecal *Lactobacillus* results, there was only a slight linear effect on *Lactobacillus* at week 6 (7.03 *vs* 7.04, 7.16 and 7.14), so future research is necessary to confirm whether 1,3-DAG has an effect on improving lactobacilli in the animal's gut.

In conclusion, dietary supplementation with graded levels of 1,3-DAG exerted positive effects on growth performance, the ATTD of gross energy and fecal microbiota. These findings may provide an insight into a new nutritional strategy for growing pigs.

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คำสำคัญ: 1,3-diacylglycerol growing pigs growth promoter

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