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Growth Performance, Blood Lipid Profile, Relative Organ Weight and Meat Quality of Broilers Fed Corn-Soybean Meal Based Diets Containing Graded Levels of 1,3-Diacylglycerol

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IHK conceived and designed the study. WCL and KSY performed experimental work and laboratory analysis. KSY helped in preparation of the manuscript. WCL analyzed the data and wrote the article.

Key words
1,3-diacylglycerol, Broilers, Growth performance, Serum lipid profile.

ABSTRACT

This 28-d feeding trial was conducted to evaluate the effects of dietary supplementation with different levels of 1,3-diacylglycerol (1,3-DAG) on growth performance, blood lipids profiles, relative organ weight and meat quality of broilers. A total of 512 male, one-d-old Ross 308 broilers with an average initial BW of 45.28 ± 0.81 g were randomly allotted to 4 treatments. Each dietary treatment consisted of 8 replicate cages, with 16 birds per replicate. The 4 dietary treatments were corn-soybean meal-based diets and supplemented with 0, 0.05, 0.10 and 0.15% 1,3-DAG, respectively. During d 1-14, with increasing the dietary 1,3-DAG levels from 0 to 0.15%, the body weight gain (BWG) was improved (linear, P<0.01), whereas the feed conversion ratio (FCR) was decreased (linear, P<0.01). During d 14-28, dietary supplementation of graded level of 1,3-DAG improved the BWG (linear, P<0.05) and decreased the FCR (linear, P<0.01). During d 1-28, there was a linear increase in BWG (P<0.01) and a linear decrease in FCR (P<0.01) with increasing 1,3-DAG supplementation. In addition, dietary 1,3-DAG supplementation tended to increase the serum HDL cholesterol levels (quadratic, P=0.074). Furthermore, the relative weight of bursa of Fabricius was increased by supplementation with 1,3-DAG (quadratic, P<0.05). In conclusion, dietary supplementation of different levels of 1,3-DAG in broilers diets could improve the growth performance, modify blood lipid profile and promote the development of bursa of Fabricius.

INTRODUCTION

Fat is recognized as a high calorie dense ingredient and considerable energy source in poultry nutrition, however, previous studies in broilers have shown that birds do not utilize and absorb fats effectively, in particular, the young broilers (Blanch *et al*., 1995; Baião and Lara, 2005). This may be due to a rapid feed transit time and low levels of bile salt and pancreatic secretions (Noy and Sklan, 1995; Meng *et al*., 2004; Sultan *et al*., 2018). Emulsifiers can help to reduce the surface tension and increase the formation of emulsion droplets, stimulate the formation of micelles, promote the concentration of monoglycerides in the intestine, and facilitate the nutrient transport through the membrane, thus allowing a better nutrient absorption and utilization (Melegy *et al*., 2001; Siyal *et al*., 2017).

In the available literature, it has been reported that the application of emulsifiers in broilers diets could improve the growth performance and feed efficiency by increasing the digestibility and absorption of fats, and also modify the blood lipids (San Tan *et al*., 2016; Wang *et al*., 2016; Zhao and Kim, 2017; Bontempo *et al*., 2018). However, others authors have reported that supplementation of emulsifiers showed less effects on growth performance and blood lipid profile of broiler chicks (Roy *et al*., 2010; Aguilar *et al*., 2013). These responses probably due to the different chemical characteristics of the used emulsifiers.

The diacylglycerol (DAG), 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 principal function of DAG is related to their amphiphilic nature and surface-active properties, being well-known as emulsifier ingredients in food industry (Martin *et al*., 2014). In this regards, DAG has been used in synergistically with monoacylglycerol as emulsifier to improve the fat digestibility (Flickinger

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and Matsuo, 2003; Shimada and Ohashi, 2003; Nakajima, 2004). Supportably, recent evidence demonstrated that 1,3-DAG could be used as emulsifier and supplementation with 1,3-DAG in low energy diets improved the growth performance and crude fat digestibility of broilers (Upadhaya *et al*., 2017). On the other hand, the DAG can be absorbed directly into the blood and enter hepatocytes via the portal vein for rapid oxidation due to the reduced chain length (Murata *et al*., 1997; Murase *et al*., 2002). However, no research has been reported to study the effect of supplementing graded levels of 1,3-DAG in broilers fed basal diets. Therefore, the present experiment was conducted to evaluate the effectiveness of dietary 1,3-diacylglycerol (DAG) supplementation for broilers fed corn-soybean meal based diets.

MATERIALS AND METHODS

All experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea.

Birds, diets and experimental procedures

A total of 512 male, one-d-old Ross 308 broilers with an average initial BW of 45.28 ± 0.81 g were randomly allotted to 4 treatments. Each dietary treatment consisted of 8 replicate cages, with 16 birds per replicate. All birds were raised in stainless steel pens with concrete floors covered with clean rice bran. The ingredient composition and nutrient content of basal diets for both experimental phases are presented in Table I. Basal diets were formulated to meet or exceed Ross 308 catalogue recommendations (Anonymous, 2007). The 4 dietary treatments were corn-soybean meal-based diets and supplemented with 0, 0.05, 0.10 and 0.15% 1,3-DAG, respectively. The 1,3-DAG (50%) mixed with carrier was obtained from the commercial company (Il Shin Wells, Korea). All experimental diets were given to the birds in mash form. Birds had free access to feed and water. Lighting was continuous, and room temperature was maintained at 33 ± 1 °C for the first 3 d, and then gradually reduced by 3 °C a week until reaching 24 °C and maintained for the remainder of the experiment.

Sampling and measurements

Broilers were weighed on a cage basis on d 0, 14 and 28, and feed consumption was recorded throughout the experiment. The body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were then calculated using this information for each phase.

At the end of the experiment, 8 birds (1 bird per cage) were randomly selected from each treatment and about 5 ml of blood samples were collected from the wing vein using sterile syringe and then transferred into vacuum tubes (anticoagulant-free, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). After collection, the serum samples from vacuum tubes were centrifuged (2,000 × g) for 30 min at 4 °C. The concentration of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and total cholesterol in the serum samples were analyzed with a commercial kit (Sigma Diagnostics, MO, USA) according to the manufacturer's protocol.

Table I.- Basal diet composition (as-fed basis).

Item	Day 1-14	Day 15-28
Ingredients (%)		
Corn	56.95	60.44
Soybean meal (CP 45%)	29.25	25.33
Corn gluten meal (CP 60%)	4.44	3.83
Tallow	3.61	5.00
Limestone	0.91	1.02
Dicalcium phosphate	2.07	1.93
Salt	0.32	0.37
Methionine (99%)	0.33	0.37
Lysine-HCI (24%)	1.68	1.28
Threonine (98.5%)	0.18	0.18
Vitamin premix[1]	0.06	0.05
Trace mineral premix[2]	0.10	0.10
Choline (50%)	0.10	0.10
Calculated values		
ME (kcal/kg)	3050	3200
CF (%)	6.10	7.50
Lys (%)	1.40	1.20
CP (%)	22.25	20.47
Met (%)	0.63	0.64
Met+Cys (%)	1.38	1.41
Analyzed values		
GE (kcal/kg)	3679	3840
CF (%)	6.20	7.53
CP (%)	21.10	19.25
Ca (%)	0.90	0.90
Total P (%)	0.71	0.66

[1]Provided per kilogram of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 µg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of pantothenic acid. [2]Provided per kilogram of diet: 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, and 62.5 mg of S.

Table II.- Effects of dietary supplementation with graded levels of 1,3-diacylglycerol (DAG) on growth performance in broilers.

Items[1]	Dietary 1,3-DAG levels (%)				SEM[2]	P-value		
	0	0.05	0.10	0.15		Linear	Quadratic	Cubic
Day 1-14								
BWG (g)	417	419	431	432	4	0.004	0.938	0.284
FI (g)	492	483	475	476	7	0.072	0.441	0.748
FCR	1.179	1.153	1.102	1.103	0.020	0.005	0.509	0.396
Day 15-28								
BWG (g)	1085	1099	1102	1142	16	0.023	0.421	0.503
FI (g)	1663	1637	1627	1646	18	0.458	0.216	0.873
FCR	1.533	1.493	1.479	1.445	0.021	0.007	0.901	0.628
Overall (Day 1-28)								
BWG (g)	1502	1518	1533	1574	17	0.007	0.478	0.716
FI (g)	2155	2120	2101	2122	20	0.201	0.169	0.796
FCR	1.434	1.399	1.373	1.349	0.016	0.001	0.709	0.926

[1]BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio. [2]Standard error of means.

For determining the breast meat quality, 8 birds from each treatment (1 bird per replication cage) were killed by cervical dislocation after completion of bleeding from the jugular vein. The gizzard, breast meat, bursa of Fabricius, liver, and abdominal fat were then removed by trained personnel and weighed and analyzed immediately. Organ weight was expressed as a percentage of BW. The breast muscle Hunter lightness (L*), redness (a*), and yellowness (b*) values were determined using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Duplicate pH values of breast muscle for each sample were measured using a pH meter (Fisher Scientific, Pittsburgh, PA). The water holding capacity (WHC) was measured based on the methods described by Kauffman *et al*. (1986). Briefly, a 0.3 g sample was pressed at 3,000 psi for 3 min on a 125-mm-diameter piece of filter paper. The areas of the pressed sample and the expressed moisture were delineated and then determined using a sensor (Digitizing Area Line Sensor, MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water:meat area was calculated, giving a measure of WHC (a smaller ratio indicates increased WHC). Drip loss was measured using approximately 2 g of meat sample according to the plastic bag method described by Honikel (1998).

Statistical analysis

All data were analyzed using mixed procedures of SAS (SAS Institute Inc., Cary, NC). The model used was $Y_{ijk} = \mu + t_i + r_k + e_{ijk}$, where Y_{ijk} is an observation on the dependent variable *ij*, μ is the overall population mean, t_i is the fixed effect of 1,3-DAG addition treatments, r_k is the pen as a random effect, and e_{ijk} is the random error associated with the observation *ijk*. In addition, orthogonal polynomial contrasts were used to test the linear, quadratic and cubic effects of increasing levels of 1,3-DAG. Variability in the data was expressed as standard error of means (SEM) and P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Growth performance

As described in Table II, during d 1-14, with increasing the dietary 1,3-DAG levels from 0 to 0.15%, the BWG was improved (linear, P<0.01), whereas the FCR was decreased (linear, P<0.01). During d 14-28, dietary supplementation of graded level of 1,3-DAG improved the BWG (linear, P<0.05) and decreased the FCR (linear, P<0.01). During d 1-28, there was a linear increase in BWG (P<0.01) and a linear decrease in FCR (P<0.01) with increasing 1,3-DAG supplementation.

The digestive physiology of young chicks is characterized by low levels of natural endogenous lipase production. Therefore, supplementation of emulsifiers, particularly at early stages of developmental growth, allows the chicks to improve digestion and absorption of the fats in order to increase energy concentration and growth performance (San Tan *et al*., 2016). The results from the present study showed that dietary supplementation of 1,3-DAG as a emulsifier could improve growth performances (BWG and FCR) of broilers. These data are in agreement with the findings of the reports from Upadhaya *et al*. (2017), who demonstrated that addition of 1,3-DAG as fat emulsifier in low energy diet promoted the growth rate of broilers. Roy *et al*. (2010) also reported that supplementation of exogenous emulsifiers improved broiler growth performance. Bontempo *et al*. (2018) confirmed that supplementation with a synthetic emulsifier improved the ADG of broiler chicks. These findings may be ascribed to emulsifiers reduced the surface tension of water, increase penetration and improve the distribution of water in press meal. Moreover, it was suggested that the addition of DAG helped in slowing down the rate of feed passage through the digestive tract, thus allowing better absorption and digestion of nutrients present in the diet (Sayed, 2009). These activities would benefit the growth rate of broilers (Siyal *et al*., 2017). Furthermore, the DAG can be absorbed directly into the blood and enter hepatocytes via the portal vein for rapid oxidation (Yanai *et al*., 2007), consequently, improved the energy utilization and rapidly supplied energy for growth.

Serum lipid profile

As shown in Table III, dietary supplementation with different levels of 1,3-DAG tended to increase the serum HDL-C concentration (quadratic, P=0.074). It has been recognized that a higher blood HDL cholesterol concentration were beneficial for the health of animals (Brewer, 2004). The results of this study suggested that dietary 1,3-DAG supplementation exerted partial effects on increasing serum HDL cholesterol levels. Similarly, Roy *et al*. (2010) reported that exogenous emulsifier (polyethylene glycol ricinoleate) modified blood lipids profiles, such as decreased total cholesterol and LDL-C level, but exerted no effect on HDL-C of broilers at the age of d 20. A study conducted by Zhao and Kim (2017), showed decreased concentrations of total and LDL cholesterol and triglycerides, but not HDL, in the emulsifier (lysophospholipids) treated chicks compared to the control group. The authors suggested that the response could be related to the age of broilers and also to the inclusion level. On the contrary, Wang *et al*. (2016) did not observe that dietary emulsifier (sodium stearoyl-2-lactylate) had any effect on serum total triglycerides, cholesterol, LDL-C and HDL-C levels of broilers fed a low energy diet. The similar findings in broilers were detected by Aguilar *et al*. (2013). It has been also reported that the serum lipid concentrations in humans were not affected after treatment with diacylglycerol oil (Yuan *et al*., 2010). The observations of this study did not fully support other reports in human subjects and avian species may be due to the inclusion level and the chemical characteristics of emulsifiers.

Relative organ weight

The effects of 1,3-DAG on relative organ weight were presented in Table IV. The relative weight of bursa of Fabricius was increased by supplementation with various levels of 1,3-DAG (quadratic, P<0.05). The bursa of Fabricius is very important central immunity organ, and is the humoral immunity center in poultry. The results of this study indicated the 1,3-DAG may possess immunomodulatory properties. However, few researchers investigated the effects of emulsifier or 1,3-DAG supplementation on the bursa of Fabricius and immunity in avian species and the results are not univocal. 1,3-DAG is hydrolyzed to 1,3-monolaurin by lipase *in vivo*. The monolaurin, has profound antiviral and antibacterial activity (Lieberman *et al*., 2006). Research has suggested that monolaurin exerts virucidal and bactericidal effects by solubilizing the lipids and phospholipids in the envelope of the pathogen causing the disintegration of its envelope (Projan *et al*., 1994). The antiviral and antibacterial activity of monolaurin may be responsible for the improved immune response and development of immune organ. However, the underlying mechanism is not quite clear yet and requires further study.

Breast meat quality

The present study suggested that dietary 1,3-DAG supplementation tended to decrease the drip loss at d 5 (Table V; quadratic, P<0.10). In previous studies, it is reported that chicken muscle drip loss could be decreased by adding 1,3-DAG (Upadhaya *et al*., 2017). That is partly in agreement with the finding of current study. The ability of muscle proteins to attract water and hold it within the cells is of paramount importance to meat quality. Moreover, the correlation between meat quality and oxidation resistance of muscle has been well documented (Downs *et al*., 2000). It is assumed that 1,3-DAG may

Table III.- Effects of dietary supplementation with graded levels of 1,3-diacylglycerol (DAG) on blood lipids profiles in broilers.

Items[1] (mg/dL)	Dietary 1,3-DAG levels (%)				SEM[2]	P-value		
	0	0.05	0.10	0.15		Linear	Quadratic	Cubic
HDL-C	92.15	88.31	88.70	103.05	5.22	0.154	0.074	0.643
LDL-C	22.10	26.42	24.83	24.26	2.40	0.780	0.292	0.246
Total cholesterol	125.38	127.30	127.29	128.80	7.45	0.707	0.907	0.917

[1]HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. [2]Standard error of means.

Table IV.- Effects of dietary supplementation with graded levels of 1,3-diacylglycerol (DAG) on relative organ weight in broilers.

Items (%)	Dietary 1,3-DAG levels (%)				SEM[1]	P-value		
	0	0.05	0.10	0.15		Linear	Quadratic	Cubic
Liver	2.60	2.93	2.76	2.94	0.15	0.220	0.627	0.221
Bursa of Fabricius	0.15	0.18	0.18	0.16	0.01	0.472	0.026	0.810
Breast muscle	17.56	18.25	18.84	18.52	0.73	0.301	0.499	0.808
Abdominal fat	1.35	1.23	1.21	1.31	0.09	0.724	0.226	0.963
Gizzard	0.89	0.88	0.90	0.88	0.06	0.973	0.974	0.778

[1]Standard error of means.

Table V.- Effects of dietary supplementation with graded levels of 1,3-diacylglycerol (DAG) on breast meat quality in broilers.

Item	Dietary 1,3-DAG levels (%)				SEM[1]	P-value		
	0	0.05	0.10	0.15		Linear	Quadratic	Cubic
pH value	5.47	5.44	5.41	5.47	0.03	0.886	0.168	0.609
Breast muscle color								
Lightness (L*)	55.07	55.36	52.11	55.83	1.81	0.907	0.353	0.206
Redness (a*)	15.20	15.05	14.61	15.08	0.46	0.699	0.510	0.572
Yellowness (b*)	8.73	8.85	8.34	8.71	0.55	0.825	0.820	0.551
Water-holding capacity (%)	61.48	61.11	62.31	61.59	0.63	0.598	0.786	0.230
Drip loss (%)								
1 d	3.09	2.46	2.86	2.64	0.45	0.639	0.646	0.406
3 d	7.39	6.85	6.67	7.73	0.49	0.707	0.119	0.693
5 d	13.20	12.16	11.49	12.43	0.57	0.251	0.097	0.631
7 d	15.55	15.80	15.94	15.52	0.38	0.989	0.391	0.806

[1]Standard error of means.

reduce lipid peroxidation in the muscles by maintaining the integrity of cell membranes, and reduced the rate of water loss (Upadhaya *et al*., 2017). This speculation would be worthy to be further investigated.

CONCLUSION

On the whole, dietary supplementation with graded levels of 1,3-DAG in broilers diets improved the growth performance and the weight of bursa of Fabricius, and showed partial positive effects on serum HDL concentration and drop loss of breast muscle. However, further research is necessary to confirm the conclusions and to illustrate the underlying mechanism.

Statement of conflict of interest

Authors have declared no conflict of interest.

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