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Effects of Emulsifier and Multi-enzyme in Different Energy Densitydiet on Growth Performance, Blood Profiles, and Relative Organ Weight in Broiler Chickens

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Abstract

This study was conducted to determine the effects of emulsifierand multi-enzyme in different energy density diet on growth performance, blood profiles, and relative organ weight in broiler chickens. A total of five hundred and forty2-d-oldmale Ross broilers (BW of 42.4 ±1.3 g) wereused in a 35-d experiment and randomly divided into 5 treatment groups: 1) NC [low energy diet, 3% tallow, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)], 2) PC [high energy diet, 5.5% soybean oil, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))], 3) P1 (NC+0.1% multi-enzyme), 4) P2 (NC + 0.05% emulsifier), 5) P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier). Multi-enzymecontained α-galactosidase, galactomannase, xylanase, and β-glucanase. Emulsifier was a commercial product named Prosol® which wassodium stearoyl-2-lactylate.There were 9 replications per treatment with 12 birds per pen. From d 0 to 21, body weight gain (BWG) in PC and P1 treatments increased ($P < 0.05$) compared with NC treatment. From d 22 to 35, feed intake (FI) was greater ($P < 0.05$) in P3 treatment than PC treatment. On d 35, triglyceride concentration in PC, P1 and P3 treatments was greater ($P < 0.05$) compared with NC treatment. No differences were observed on white blood cell (WBC), red blood cell (RBC) and glucose concentration. The relative weight of the spleen was significantly decreased ($P < 0.05$) in P3 treatment compared with PC treatment. Furthermore, the relative weight of the bursa of Fabricius in P3 treatment was higher ($P < 0.05$) than PC, P1 and P2 treatments. In conclusion, the results of this study indicate that emulsifier andmulti-enzymein low-density diets can partially improve growth performance, triglyceride, and relative organ weight in broiler chickens, which can counterpart the negative effects caused by the decreased nutrient concentration.

Keywords: cholesterol, emulsifier, multi-enzyme, fatty acid profile, broilers

1. Introduction

Sodium stearoyl-2-lactylate (SSL) is the sodium salt of a long-chained carboxylic acid containing two ester linkages. It is applied as emulsifier (Gomez et al., 2004; Manohar et al., 1999), whipping agent (Kelly et al., 1999) and conditioning agent (Armero et al., 1998) in a wide variety of modern food technologies. Emulsification is required for micellar formation and absorption of fat, so exogenous emulsifiers may enhance fat utilization fed high-fat diet. Kussaibati et al. (1982) reported that bile salts supplementation in broiler chicken diets improves emulsion formation and fat digestibility. Emulsifier promotes the incorporation of fatty acids into micelles. Augur et al. (1947) and Polin (1980) reported that digestibility of fat increased when emulsifier was mixed with the fat before it was fed to rats and chicks, respectively. Research investigating the effect of emulsifiers on performance of broiler chickens is limited, so further researches need to be taken. Therefore, with the current high prices of feed ingredients, nutritional emulsifiers may help in reducing feed costs if beneficial effect was observed in broiler chickens.

The presence of galactomannan in the diet has been shown to diminish the growth of broilers (Ray et al. 1982). Use of exogenous enzymes may also provide opportunities to utilize α-1, 6-galactosides andβ-galactomannan as energy sources (Kim et al., 2003; Wang et al., 2009; Ao et al., 2011). Multi-enzyme contains α-galactosidase, galactomannase, xylanse and β-glucanase. The α-galactosides of sucrose can't be broken down in the small intestine of chickens or pigs due to the absence of endogenous α-(1,6)-galactosidase (Gitzelmann et al., 1965). Exogenous α-galactosidase enzymes supplementation for chickens has led to variable results (Ghazi et al., 1997;

Ghazi et al., 2003; Graham et al., 2002; Knap et al., 1996; Kidd et al., 2001a,b). Waldroup et al. (2005) reported that in their previous study, they were unable to detect an improvement in the energy value of soybean meal as a result of addition of α-galactosidase enzyme. Until now, the effect of multi-enzyme on growth performance in broiler chickens remains unclear.

The principal objective of this study was to evaluate effects of emulsifier and multi-enzyme on growth performance, blood profiles, and relative organ weight in broiler chickens.

2. Materials and Methods

All broilers used in this trial were handled in accordance with the guidelines set forth by the Animal Care and Use Committee of Dankook University.Sodium stearoyl-2-lactylate (Prosol®) which was supplied by Il Shin WellsCompany (Seoul, South Korea).

2.1 Enzyme Preparation

Multi-enzyme preparation (Endopower®, EasyBio System Incorporated, Seoul, South Korea) contains 7 unit/g α-galactosidase activity, 22 unit/g galactomannanase activity, 300 unit/g xylanase activity and 220 unit/gβ-glucanase activity. One unit of α-galactosidase is defined as the amount of enzyme that liberates 0.1 μmol nitro phenol from 2 mmol of pNPG (p-nitrophenyl-alpha-dgalactoside) per at 30°C and pH 4.0. One unit of galactomannase is defined as the amount of enzyme that liberates 0.1 μmol total reducing sugars/min from 0.5% galactomannan per at 30°C and pH 4.0. One unit of xylanase is defined as the amount of enzyme that liberates 1 mg total reducing sugar/10 min. from 0.5% xylan at 30°C and pH 4.0. One unit of β-glucanase is defined as the amount of enzyme that liberates 1 mg of total reducing sugar per 10 min from 0.4% β-glucan at 30°C and pH 4.0.

2.2 Experimental Design, Animals, and Housing

A total of five hundred and forty2-d-old male Ross broiler chickens (BW of 42.4 ± 1.3 g) were used in a 35-d experiment. Broiler chickens were allotted into 5 treatments and there were 9 replications per treatment with 12 birds per pen.Dietary treatment groups include: 1) NC [low energy diet, 3% tallow, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)], 2) PC [high energy diet, 5.5% soybean oil, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))], 3) P1 (NC + 0.1% multi-enzyme), 4) P2 (NC + 0.05% emulsifier), 5) P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier).The basal diets were formulated to meet or exceed the NRC (1994) nutrient requirements. Broiler chickens were raised in a temperature-controlled room with stainless steel pens of identical size (1.75 × 1.55m). Room temperature began at 33°C from d 1 to 3 and was reduced gradually to 24°C until the end of the experiment and the relative humidity was around 60%.Broiler chickens received diet and water *ad libitum*. Each pen had a pan feeder with a 35-cm diameter. Water was provided by evenly spaced nipple drinkers (5 nipples per pen) positioned along the side wall of the pen.

2.3 Sampling and Measurements

All diets were grounded through a 1-mm screen in a Wiley mill before analyzing for crude protein (CP), calcium (Ca), and phosphorus (P) (AOAC, 2003). N content in diets was determined by using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Amino acids (excluding tryptophan) in diets were analyzed by dansylation (Beckman Intruments Inc., Fullerton, CA) and HPLC after acid hydrolysis for 24h in 6 *M* HCl.

The broiler chickens were weighed by pen and feed intake (FI) was recorded on d 0, 7, 21, and 35 to calculate body weight gain (BWG) and feed conversion ratio (FCR). At the end of the experiment, 45 broiler chickens were randomly selected from each treatment (5 birds per pen) and blood samples were collected from the wing vein into a sterile syringe and stored at −4°C. The white blood cell (WBC), red blood cell (RBC), and lymphocyte counts in the whole blood were then determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY). Samples for serum analysis were then centrifuged at 3,000×g for 15 minutes at 4°C. The concentrations of glucose and triglyceride in the serum samples were analyzed with an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) using colorimetric methods.After blood collection, the same broiler chickens were weighed individually and slaughtered by cervical dislocation. The liver, spleen, bursa of Fabricius, breast meat, abdominal fat, and gizzard were then removed by trained personnel and weighed.To avoid variation in the cutting procedures, the same operator was employed. Organ weight was expressed as a percentage of BW.

2.4 Statistical Analysis

All experimental data was analyzed in accordance with the GLM Procedure (SAS, 1996). The pen was used as the experimental unit.Differences among treatment means were determined using the Duncan's multiple range test. A probability level of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Growth Performance

From d 0 to 21, BWG of broiler chickens fed the PC and P1 diets increased ($P < 0.05$) compared with broiler chickens fed the NC diet (Table 2). Feed intake (FI) and feed conversion ratio (FCR) of broiler chickens weren't affected by any treatment diets. From d 22 to 35, FI was greater ($P < 0.05$) in P3 treatment than PC treatment and there were no differences on BWGand FCR. During the overall experiment, no significant differences in BWG, FI, and FCR were observed among the dietary treatments.

3.2 Blood Profiles

On d35, triglyceride concentration of broiler chickenblood fed PC, P1 and P3 diets was greater ($P < 0.05$) compared with NC diet (Table 3). In our study, there were no differences in WBC, RBC and glucose concentration in broiler chicken blood.

3.3 Relative Organ Weight

The relative weight of the spleen was significantly decreased ($P < 0.05$) in P3 treatment compared with PC treatment (Table 3). Furthermore, the relative weight of the bursa of Fabricius in P3 treatment was higher ($P < 0.05$) than PC, P1 and P2 treatments. The relative weight of the liver, breast meat, abdominal fat and gizzard remained unaffected by any treatment diets.

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

	Starter[1]		Finisher[1]	
Item	NC	PC	NC	PC
Ingredients (%)				
Corn	49.92	55.30	58.87	67.33
Soybean meal (CP 48%)	30.25	28.25	24.61	20.61
Corn gluten meal (CP 60%)	6.50	6.50	3.50	3.50
Rice rye	6.00	-	6.00	-
Soybean oil	-	5.50	-	4.50
Tallow	3.00	-	3.00	-
Dicalcium phosphate	2.46	2.46	2.29	2.29
Limestone	0.89	0.89	0.75	0.75
Salt	0.20	0.20	0.20	0.20
$_{DL}$-Methionine (98%)	0.17	0.29	0.17	0.21
$_{L}$-Lysine-HCl (78%)	0.21	0.21	0.21	0.21
Vitamin premix[2]	0.20	0.20	0.20	0.20
Trace mineral premix[3]	0.20	0.20	0.20	0.20
Calculated composition, %				
ME, kcal/kg	3100.00	3250.00	3025.00	3175.00
CP	22.00	22.00	19.00	19.00
Lysine	1.10	1.10	1.00	1.00
Ca	1.00	1.00	0.90	0.90
Available P	0.80	0.80	0.75	0.75
Met+Cys	0.89	0.89	0.84	0.84
Analytical composition, %				
CP	22.30	22.30	20.10	20.10
Ca	1.00	1.00	0.91	0.91
Met+Cys	0.88	0.88	0.85	0.85
Available P	0.45	0.45	0.40	0.40

[1]Starter diets, provided during week 0 to 3; grower diets, provided during week 4 to 5.

[2]Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D3, 37.5 mg of vitamin E, 2.55 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 24 μg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.

[3]Provided per kg of diet: 37.5 mg Zn (as $ZnSO_4$), 37.5 mg of Mn (MnO_2), 37.5 mg of Fe (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 0.83 mg of I (as KI), and 0.23mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

Table 2. Effects of emulsifier and multi-enzyme on growth performance in broiler chickens[1]

Items	NC	PC	P1	P2	P3	SE[2]
Starter phase (d0to d21)						
BWG, g	856[b]	956[a]	977[a]	927[ab]	919[ab]	29
FI, g	1,441	1,494	1,501	1,430	1,484	56
FCR	1.683	1.563	1.536	1.543	1.615	0.10
Grower phase (d22 to d35)						
BWG, g	980	882	853	884	950	50
FI, g	1,722[ab]	1,624[b]	1,673[ab]	1,660[ab]	1,812[a]	52
FCR	1.757	1.841	1.961	1.877	1.907	0.09
Overall the experiment (d0 to d35)						
BWG, g	1,837	1,839	1,831	1,811	1,869	50
FI, g	3,163	3,118	3,174	3,090	3,296	67
FCR	1.722	1.695	1.733	1.706	1.763	0.04

[1]Abbreviation: NC [low energy diet, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)]; PC [high energy diet, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))]; P1 (NC + 0.1% multi-enzyme); P2 (NC + 0.05% emulsifier); P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier.

[2]Standard error.

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

Table 3. Effects of emulsifier and multi-enzyme on blood profiles in broiler chickens on d 35[1]

Items	NC	PC	P1	P2	P3	SE[2]
WBC,10^3/ul	409	393	434	407	412	23.1
RBC,10^6/ul	2.24	2.33	2.30	2.24	2.25	0.07
Glucose, mg/dl	245	272	248	251	251	14.7
Triglyceride, mg/dl	58.8[c]	107.8[a]	101.5[a]	77.5[bc]	87.8[ab]	6.89

[1]Abbreviation: NC [low energy diet, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)]; PC [high energy diet, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))]; P1 (NC + 0.1% multi-enzyme); P2 (NC + 0.05% emulsifier); P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier.[2]Standard error.

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

Table 4. Effects of emulsifier and multi-enzyme on the relative organ weightof broiler chickens[1]

Items, %	NC	PC	P1	P2	P3	SE[2]
Liver	2.38	2.24	2.10	2.47	1.76	0.22
Spleen	0.153[ab]	0.206[a]	0.102[ab]	0.136[ab]	0.079[b]	0.33
Bursa of Fabricius	0.168[ab]	0.142[b]	0.143[b]	0.153[b]	0.285[a]	0.04
Breast meat	15.88	15.92	15.30	15.00	16.74	0.98
Abdominal fat	1.50	2.20	2.34	1.84	1.62	0.35
Gizzard	1.56	1.61	1.44	1.38	1.44	0.13

[1]Abbreviation: NC [low energy diet, ME = 3000 (1 to 21 d) and 3100 kcal/kg (22 to 35 d)]; PC [high energy diet, ME = 3150 (1 to 21 d) and 3250 kcal/kg (22 to 35 d))]; P1 (NC + 0.1% multi-enzyme); P2 (NC + 0.05% emulsifier); P3 (NC + 0.1% multi-enzyme + 0.05% emulsifier.

[2]Standard error.

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

4. Discussion

The results of this study indicated that the low energy density diet (150 kcal/kg ME lower) as a result of addition 6% rice rye and lower fat addition (tallow 3.0 % vs. 5.5% soybean oil) can reduce ADG from d 1 to 21 and increase ADFI from d 21 to 35. In general, the lower energy (ME) level can result in a depression of growth performance. Broilers had the ability to regulate feed intake based on the energy levels of the diet, however this effect was limited during the first week of age, especially if feed the mash feed (Jones & Wiseman, 1985; Scott et al., 2002). The reason may be mainly attributed to the capacity limitation of the gastro-intestine of chicks.

Soybean meal contains α-1,6-galactosidic oligosaccharides (Wang et al., 2005). These α-galactosides have been implicated in reducing energy utilization, fiber digestion, and feed retention in soybean meal fed chickens (Coon et al., 1990). Feed enzymes have been added to poultry feed mainly to improve energy utilization in diet with high soluble non-starch-polysaccharides levels (Yu et al., 2007). The supplemented enzyme breaks the polymeric chains of the non-starch polysaccharides into smaller units thus improving the nutritive value of the feed ingredient (Smits & Annison, 1996; Kwon et al., 2003).

Sodium stearoyl-2-lactylate (SSL) is a useful emulsifier with a very high hydrophilic-lipophilic balance in the manufacture of baked goods for many reasons and is therefore an excellent emulsifier for fat-in-water emulsions. The high emulsifying efficiency of SSL is based on its amphiphilic nature consisting of a hydrophilic charged head and long hydrophobic hydrocarbon tail.SudiptoHalda and T. K. Ghosh (2010) reported that reduction in dietary energy content may result in significant improvement in feed conversion by lowering the feed intake under the influence of nutritional emulsifiers. These improvements in FCR indicate the nutritional emulsifiers compensate for an energy reduction in broiler diets without reducing growth parameters. Jones et al. (1992) concluded that addition of emulsifiers increased digestibility of nutrients but had minimal effect on growth performance in weanling pigs.

Previous researches showed that the effects of mannanase and galactosidase enzymes on broiler performance had an inconsistent response. Whereas Jackson et al. (2003, 2004) and Daskiran et al. (2004) reported beneficial effects on BWG and FCR during starter and grower phases of growth from 80 or 110 unit/g β-mannanase supplementation. Similarly, Kidd et al. (2001) fed broilers corn and soybean meal based diets with or without anα-galactosidase (112 g/ton) enzyme. They noted improved feed conversion and livability from α-galactosidase supplementation in broilers. Wang et al. (2005) and Ao et al. (2009) also reported that growth performance was improved as a result of 250 mg/kg galactosidase supplementation with 90.2 unit/g enzyme activity or 0.1%galactosidase supplementation with 1724 unit/kg enzyme activity. On the other hand, Torki and Chengeni (2007) reported no improvement in broiler performance by adding more than 165×10^6 unit/kg β-mannanase into corn-soybean meal based diets. In addition, Irish et al. (1995) reported no improvement on performance in broilers by adding 0.20 g/kg α-galactosidase to a corn-soybean meal.

In our experiment, Supplementation of multi-enzyme complex and/or emulsifier (SSL) to low ME diet can increase ADG to the same levels with high ME level diet. This result indicated that multi-enzyme complex can increase the energy utilization in low energy corn-soybean basal diet. Similarly, Zhou et al. (2009) reported that multi-enzyme (Xylanase, α-amylase and protease) supplementation enhanced the ME value of diet, and improve the ADG of broilers. By preventing the formation of viscous digesta, exogenous enzymes can improve energy utilization in diet with high soluble non-starch-polysaccharides levels, such as wheat and rye (Yu et al., 2007; Zhou et al., 2009; Ao et al., 2009). Moreover, the addition of xylanase and β-glucanase can reduce the degree of bile acid deconjugation, which may also benefit the fat utilization and improve the growth performance in low density diet (Mathlouthi et al., 2002).

Multi-enzyme provided β-1,4-mannanase, xylanase, β-glucanase, and α-1,6-galactosidase (Wang et al., 2009; Ao et al., 2011). Despite the presence of these enzymes, no significant improvements though energy in the basal diet of P1 and P2 treatments was lower compared with PC treatment, there was no difference on growth performance, blood profile, and relative organ weight in PC, P1, and P2 treatments. It indicated that SSL increased the energy utilization, so growth performance, blood profile, and relative organ weight of broiler chickens in PC, P1, and P2 treatments reached the same level. Triglyceride in P2 treatment decreased significantly, and it meant that SSL broke down the triglyceride to energy use.Wieland et al. (1993) also showed that the effectiveness of different emulsifiers in increasing the absorption of medium-chain triglycerides varied. From the difference between P1 and P2 treatment, we knew that SSL would break down more triglyceride if the energy of basal diet was lower. Praharaj et al. (1997) reported that the developments of bursa of Fabricius and spleen in broiler chicks were not influenced by dietary energy levels (2,500, 2,600 and 2,800 kcal/kg of ME) from d 1 to 42. In our study, difference on bursa of Fabriciuswas observed between PC and P3 treatment. There was no difference between NC and P3 treatment, so the difference between PC and P3 was from enzymes and emulsifier.Because no difference on bursa of Fabricius wasdetected in PC, P1 and P2 treatment, we could conclude that enzymes

increased the relative weight of bursa of Fabricius. It was good for immunity of broiler chickens when multi-enzyme was added into the diet.

Although no difference was observed among treatments during the overall period, we found significant difference on BWG in NC, PC and P1 treatments from d 0 to 21. In conclusion, the results of this study indicate that emulsifier and multi-enzyme in low-density diets can partially improve growth performance, triglyceride, and relative organ weight in broiler chickens, which can counterpart the negative effects caused by the decreased nutrient concentration.

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