Evaluation of adding sodium stearoyl-2-Lactylate to energy-reduced diets on broilers’ development, nutritional digestibility, bacterial count in the excreta, and serum lipid profiles

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ABSTRACT
The purpose of this study was to evaluate the outcome of sodium stearoyl-2-lactylate (SSL) supplementation to energy reduced diets on broilers’ growth performance, apparent nutrient digestibility, excreta bacterial counts, and blood lipid profile. A total of 700 one-day-old male (feather sexed) Ross 308 broiler chickens having an average body weight of 43 ± 1.2 g were randomly allotted to five (5) dietary treatments (7 pens/treatment). The treatments consisted of: (1) positive control (PC) (basal diet with metabolisable energy, ME = 3000 kcal/kg starter; 3100 kcal/kg grower; 3200 kcal/kg finisher), (2) negative control (NC) (PC/C0 = 50 kcal/kg), (3) SSL1, NC + 0.03% SSL, (4) SSL2, NC + 0.04% SSL, (5) SSL3, NC + 0.05% SSL. The PC group showed higher BWG (body weight gain) and lower FCR (feed conversion ratio) than the NC group during days 1 to 7, and 7 to 21. SSL supplemented groups showed higher BWG and better FCR than the NC group, along with a linear increase in overall BWG was observed with SSL doses. Excreta microbial counts were not influenced by energy levels or SSL doses. In blood serum parameters, LDL (low density lipoprotein) cholesterol was reduced with a linear tendency by increasing levels of SSL supplementation. In conclusion, sodium stearoyl-2-Lactylate supplementation showed better growth and nutrient digestibility in broilers’ at an early age.

HIGHLIGHTS
1. Emulsifiers can help broilers to utilise dietary energy more efficiently
2. Sodium Stearoyl-2- Lactylate helped to increase energy and fat digestibility in broilers
3. As a result, growth performance was improved through Sodium Stearoyl-2-Lactylate supplementation in broilers

Introduction
To survive in a competitive market, broiler farming has to be more efficient in feeding, management, and production. For broilers requiring a high-energy diet, fats and oils have become worthwhile sources of energy (Panda et al. 2016). However, the energy obtained from fat sources has a limit on digestion in young birds (Lai et al. 2018). Despite enough supplementation of lipids in the diet, the reason for broilers’ failure to utilise enough lipids is not only limited lipase secretion but also poor emulsification capacity. Thus, exogenous emulsifiers are important to overcome this problem (Siyal et al. 2017). Limited lipase and bile secretions of young birds are causing inefficient utilisation of lipids where exogenous emulsifiers are considered to help in efficient digestion (Upadhaya et al. 2017). An emulsifier has a hydrophilic and a lipophilic part that helps to increase the active surface of fats, which increases the breakdown of fats into fatty acids and monoglycerides (Upadhaya et al. 2018). Emulsifiers help in the formation of micelles by increasing the surface area of lipids. They have shown increased lipid absorption, growth performance, and feed efficiency in broilers and pigs (Neto et al. 2011; Siyal et al. 2017). Commercially available emulsifiers include lysophospholipids, lecithin, sodium stearoyl-2-lactylate, 1, 3 –Diacyl glycerol, Tween 20, Tween 80, etc. Different studies reported their effects on nutrient digestibility, and growth performance of broilers (Roy et al. 2010; Aguilar et al. 2013; Kaczmarek et al. 2015; An et al. 2020; Wickramasuriya et al. 2020). As...
emulsifiers are capable of positively affecting the digestion of lipids, they also play an influential role in reducing triglycerides, cholesterol and low density lipoprotein (LDL) in broilers (Roy et al. 2010; Zhao and Kim 2017).

Sodium stearoyl-2-lactylate (SSL) in combinations with different emulsifiers showed improvement in the growth performance, digestibility, and lipid profiles in broilers and piglets (Upadhaya et al. 2018; Liu et al. 2020; Bai et al. 2019). In contrast, combinations and individual studies of SSL showed very limited or no effect on growing and finishing pigs’ performance (Yun et al. 2019; Yin et al. 2019; Hoque and Kim 2021). Elmen et al. (2020) showed that SSL supplementation can manipulate gut microbes and butyrate production in vitro. While individual studies on SSL in broilers are scarce, inconsistency is also present (Ali et al. 2017; Wang et al. 2016). Therefore, more studies are needed to evaluate the efficacy of SSL as an emulsifier in an energy reduced diet for broiler chickens.

Therefore, the present study was conducted to assess different doses of SSL supplementation in broilers’ growth performance, digestibility, excreta microbes and serum lipid profiles.

Materials and methods
This experiment methodology was approved (DK-1-2024) by Animal Care Committee, Dankook University, South Korea.

Tested emulsifier
The tested emulsifier (SSL) was provided by Il Shin Wells Company (Seoul, South Korea). Commercial stearic acid was first esterified with lactic acid and then neutralised with sodium salts. The final product included 33% stearic acid, 15% lactic acid and 2% sodium. SSL has a hydrophilic lipophilic balance range of 10 to 12.

Birds, housing and diets
Seven hundred 1-day-old male (feather sexed) Ross 308 chicks weighing 43 ± 1.2 g on average were randomly distributed to five treatments. Each treatment had seven replication pens where 20 birds were allotted to each pen. The birds were kept in slatted floor pens with 60% humidity and 18 hours fluorescent light facility. The room temperature was set at 33 °C at first. Then, it was reduced by 3 °C for each week until it reached 24 °C. The treatment diets were as: (1) PC, positive control (Metabolisable energy, ME = 3000 kcal/kg starter; 3100 kcal/kg grower; 3200 kcal/kg finisher), (2) NC, negative control (PC – 50 kcal/kg), (3) SSL1, NC + 0.03% SSL, (4) SSL2, NC + 0.04% SSL, (5) SSL3, NC + 0.05% SSL. The experimental period (35 days) was divided into 3 phases; 1-7 days, 8-21 days and 22-35 days. For these phases’ starter, grower and finisher diets (Table 1) were formulated according to Rostagno et al. (2011) recommendation. Feed and water were available as ad libitum.

Growth performance and apparent nutrient digestibility
Body weight gain (BWG) and feed intake (FI) were recorded and on days 7th, 21st, and 35th. FCR was calculated from BWG and FI records. At the end of the 35 days long study, apparent total tract digestibility (ATTD) of dry matter (DM), fat and energy (E) were estimated. For this, chromium dioxide (Cr2O3) was mixed at a rate of 0.2% with broiler diets as an indigestible marker from day 29th to 35th. From each pen, composite excreta were pooled and made to a representative sample. Feed samples and excreta samples were kept at −20 °C in a freezer. Before chemical analysis, excreta samples were thawed and dried in an oven (FC-610, Toyo Seisakusho Co. Ltd., Tokyo, Japan) at 70 °C for 70 hours. Feed and faecal samples were then ground and filtered through a 1 mm net screen. The samples were subjected to dry matter (DM), energy and fat analysis using the Association of official analytical chemists (AOAC 2005). Fat was estimated following the methods (954.02, AOAC, 2005). Gross energy was analysed in an oxygen bomb calorimeter (Parr 6100 Instrument Co., Moline, USA) for ATTD of energy. The concentration of chromium oxide in feed and faecal samples were measured by UV absorption spectrophotometry (Shimdazu UV-1201, Shimdazu, Kyoto, Japan) after digesting the samples with phosphoric acid-manganese sulphate and potassium bromate according to the procedures of Williams et al. (1962). The ATTD was calculated by using below mentioned formula.

\[
\text{ATTD (\%) = } \left\{ 1 - \frac{\left[ (\text{Nf} \times \text{Cd})/(\text{Nd} \times \text{Cf}) \right]}{100} \right\}
\]

whereas Nf = nutrient concentration in faeces (% DM), Cd = chromium concentration in the diet (% DM), Nd = nutrient concentration in the diet (% DM), and Cf = chromium concentration in faeces (% DM).
Excreta microbial analysis

On the 35th day, from each pen one chicken (7 chickens in total per treatment) was selected at random and by massaging the abdominal area, faecal samples were collected from the vent into a microtube and transported to the laboratory using an ice pack to maintain temperature. From each sample, one gram was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and homogenised. Following the 10-fold dilution, the prepared samples were cultured on MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), Lactobacillus medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany), and Salmonella Shigella (SS) agar plates (Becton, Dickinson and Company, USA) to isolate E. coli, Lactobacillus and Salmonella respectively. The incubation criteria for Lactobacillus was 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates and Salmonella Shigella (SS) agar plates were incubated at 37 °C for 24 hours. E. coli, Lactobacillus and Salmonella colonies were counted soon after removal from the incubator. For each sample three (3) culture plates were counted. Bacteria were calculated by a visual count of colonies that had 30 to 300 colonies per plate. The Bacterial count was expressed as $\log_{10}$ CFU for each gram sample (Lee 2014).

Lipid profile in blood

On day 34th, one bird per pen and total of seven birds per treatment were randomly selected and blood samples (5 mL) were collected from the wing vein using a syringe. The blood samples were stored in vacuum tubes (Becton Dickinson Vacutainer Systems, NJ, USA) and then centrifuged at 2300 xg for 30 min. Serum was collected and kept at −20 °C until further analysis. The serum was then analysed for lipid profile using an automatic biochemistry analyser (Hitachi 747, Hitachi, Tokyo, Japan).

Statistical analysis

All data were analysed by GLM procedure (SAS Institute, 2014) for randomised complete block design. Each pen represented an experimental unit. Variability in data was presented as the standard error of means (SEM). Pre-planned contrasts were used to test the effect of SSL supplementation. The contrasts were a)
Results

Growth performance

The results of sodium stearoyl-2-lactylate (SSL) supplementation to energy reduced diets on boilers’ growth performance are presented in Table 2. During days 1 to 7 and days 7 to 21, BWG was significantly higher (P ≤ .05) in the PC and SSL groups compared to the NC group. During days 1 to 7, the PC group showed a lower (P ≤ .05) FCR than the NC group whereas, SSL groups showed a tendency to show a reduced (P = .085) FCR compared to the NC group. The increasing level of SSL supplementation showed a linear (P = .002) increase in BWG in the early stage (d 1 to 7) and a linear increase during days 7 to 21. As a result, overall BWG presented a linear increase (P = .045) with the increasing level of SSL supplementation. Throughout the whole experiment, feed intake (FI) exhibited no difference (P > .05) between groups. Different doses of SSL presented a linear decrease in FCR during days 1 to 7 (P = .016) and days 7 to 21 (P = .010).

Apparent nutrient digestibility

The effect of SSL supplementation to energy reduced diets on broilers’ apparent total tract digestibility (ATTD) of nutrients is shown in Table 3. There was no difference (P > .05) among the treatment groups in the case of dry matter, fat, or energy digestibility. However, a trend in linear increase in fat digestibility (P = .069) and a tendency in linear improvement (P = .077) in energy digestibility were exerted with increasing doses of SSL.

Excreta microbial count

The effect of SSL supplementation to energy reduced diets on broilers’ excreta microbial count is shown in Table 4. No difference (P > .05) was observed in excreta Lactobacillus, Escherichia coli, and Salmonella counts among the treatment groups.

Blood lipid profile

The results of increasing the level of SSL supplementation to energy reduced diets are presented in Table 5. The serum cholesterol, triglyceride, HDL cholesterol, or LDL cholesterol concentrations were not significantly different among the treatment groups. However, increasing doses of SSL supplementation showed a tendency for a linear reduction (P = .067) of LDL cholesterol concentration in blood serum.

Discussion

Sodium stearoyl-2-lactylate has been tested as an emulsifier agent that was supplemented at three different levels in broilers receiving a basal diet with animal fat as an energy source. The reduction in fat supplementation caused a difference of 50 kcal energy between the PC and NC groups. This energy difference

Table 2. The effect of emulsifier supplementation on growth performance in broilers with different energy level diets.

| Items   | PC | NC | SSL1 | SSL2 | SSL3 | SEM | P-Value |
|---------|----|----|------|------|------|-----|---------|
| d 1 to 7 |    |    |      |      |      |     |         |
| BWG, g  | 120| 114| 121  | 123  | 121  | 2   | .004    |
| FCR     | 1.212| 1.313| 1.284| 1.240| 1.242| 0.021| .772    |
| d 7 to 21 |    |    |      |      |      |     |         |
| BWG, g  | 590| 559| 584  | 589  | 585  | 7   | .030    |
| FCR     | 1.442| 1.560| 1.493| 1.492| 1.474| 0.021| .284    |
| Overall |    |    |      |      |      |     |         |
| BWG, g  | 1780| 1705| 1764| 1766| 1768| 19 | .091    |
| FCR     | 1.634| 1.709| 1.682| 1.678| 1.676| 0.017| .237    |

PC vs NC b) NC vs SSL group. Polynomial contrasts were used to test the response of different levels of SSL (0%, 0.03%, 0.04%, and 0.05%) supplementation with energy reduced diet. The significance level was set as P ≤ .05, whereas P < .10 was considered as a tendency.
caused better BWG and FCR in the PC group compared to the NC group during days 1 to 7, days 7 to 21, and overall duration. SSL supplementation presented improved BWG and FCR than the NC group during days 1 to 21 which was not observed in days 21 to 35 and the overall period. As we have used increasing doses of SSL (0.03%, 0.04%, 0.05%), BWG increased and FCR reduced linearly with dose increment in the early stages of broilers. However, FI was not different even with the reduced energy or SSL supplementation. Similar results were presented by Ali et al. (2017); Wang et al. (2016) and Upadhaya et al. (2018) using 0.05% SSL, 0.05% SSL, and 1% combined emulsifier, respectively in broilers. Ali et al. (2017) found SSL effective in all stages of the experiment, which is hard to explain due to the amount of energy reduction that was not mentioned in that study. Upadhaya et al. (2018) also found that SSL was effective in all ages of broilers, but it was at a higher supplementation (1.0%) and in a combination with Tween 20. In support of our results, Wang et al. (2016) found SSL effective during days 1 to 22 in broilers.

From our results, we consider that the digestibility of nutrients was responsible for the positive changes in growth performance. In our study, apparent total tract digestibility of fat and energy showed a tendency for linear improvement at day 35, where significant differences were not found among the groups. Observing this outcome, we assume that digestibility could have been different between the groups during days 1 to 21. Animal fat sources contain more saturated fat that cannot be digested easily. In that case, emulsifiers help to increase lipid droplet formation and reduce droplet sizes. This increases the active surface area for the lipase enzyme and produces monoglycerides. Thus, the formation of smaller micelles gets increased, which helps to improve fat digestion. There is low efficacy of digesting fat in young chickens due to lack of lipase and bile salts activity (Tancharoenrat et al. 2014). However, lipase and bile supplementation have been shown to improve digestion and growth performance in broilers (Dersjant-Li and Peiskar 2005; Alzawqari et al. 2011). Thus, increasing the level of SSL supplementation reduced the FCR through increased

### Table 3. The effect of dietary emulsifier supplementation on nutrient digestibility in broilers with different energy level diets.a.

| Items       | PC     | NC     | SSL1   | SSL2   | SSL3   | SEMa | PC vs NC | NC vs SSL | Linear | Quadratic |
|-------------|--------|--------|--------|--------|--------|------|----------|-----------|--------|-----------|
| Finish      |        |        |        |        |        |      |          |           |        |           |
| Dry matter  | 74.54  | 71.67  | 73.08  | 74.16  | 75.03  | 1.41 | .471     | .351      | .290   | .844      |
| Fat         | 68.84  | 66.17  | 66.62  | 68.95  | 69.45  | 1.44 | .377     | .295      | .069   | .983      |
| Energy      | 74.94  | 72.16  | 73.73  | 73.56  | 74.63  | 1.49 | .712     | .711      | .077   | .865      |

aPC: Basal diet; NC: Basal diet - 50 kcal; SSL: NC + 0.03% sodium stearoyl-2-lactylate; SSL2: NC + 0.04% sodium stearoyl-2-lactylate; SSL3: NC + 0.05% sodium stearoyl-2-lactylate. Data represent 1 sample/pen, 7 pen/treatment.

bStandard error of means.

cLevel of significance $p < .05$.  

### Table 4. The effect of dietary emulsifier supplementation on Microbial in broilers with different energy level diets.a.

| Items         | PC     | NC     | SSL1   | SSL2   | SSL3   | SEMa | PC vs NC | NC vs SSL | Linear | Quadratic |
|---------------|--------|--------|--------|--------|--------|------|----------|-----------|--------|-----------|
| Finish        |        |        |        |        |        |      |          |           |        |           |
| Lactobacillus | 7.48   | 7.37   | 7.47   | 7.41   | 7.47   | 0.08 | .829     | .784      | .494   | .806      |
| E.coli        | 6.06   | 6.29   | 5.88   | 6.29   | 5.98   | 0.17 | .317     | .102      | .398   | .732      |
| Salmonella    | 5.37   | 5.74   | 5.46   | 5.59   | 5.38   | 0.12 | .124     | .156      | .182   | .739      |

aPC: Basal diet; NC: Basal diet - 50 kcal; SSL: NC + 0.03% sodium stearoyl-2-lactylate; SSL2: NC + 0.04% sodium stearoyl-2-lactylate; SSL3: NC + 0.05% sodium stearoyl-2-lactylate. Data represent 1 sample/pen, 7 pens/treatment.

bStandard error of means.

cLevel of significance $p < .05$.  

### Table 5. The effect of dietary emulsifier supplementation on blood profile in broilers with different energy level diets.a.

| Items         | PC     | NC     | SSL1   | SSL2   | SSL3   | SEMa | PC vs NC | NC vs SSL | Linear | Quadratic |
|---------------|--------|--------|--------|--------|--------|------|----------|-----------|--------|-----------|
| Finish        |        |        |        |        |        |      |          |           |        |           |
| Cholesterol   | 118    | 115    | 111    | 112    | 115    | 4    | .627     | .746      | .986   | .305      |
| Triglyceride  | 27     | 29     | 27     | 27     | 27     | 2    | .762     | .609      | .363   | .355      |
| HDL cholesterol | 80    | 79     | 82     | 86     | 78     | 4    | .792     | .606      | 1.0    | .235      |
| LDL cholesterol | 31    | 31     | 26     | 25     | 23     | 5    | .280     | .269      | .067   | .558      |

aPC: Basal diet; NC: Basal diet - 50 kcal; SSL: NC + 0.03% sodium stearoyl-2-lactylate; SSL2: NC + 0.04% sodium stearoyl-2-lactylate; SSL3: NC + 0.05% sodium stearoyl-2-lactylate. Data represent 1 sample/pen, 7 pen/treatment.

bStandard error of means.

cLevel of significance $p < .05$.  

From our results, we consider that the digestibility of nutrients was responsible for the positive changes in growth performance. In our study, apparent total tract digestibility of fat and energy showed a tendency for linear improvement at day 35, where significant differences were not found among the groups. Observing this outcome, we assume that digestibility could have been different between the groups during days 1 to 21. Animal fat sources contain more saturated fat that cannot be digested easily. In that case, emulsifiers help to increase lipid droplet formation and reduce droplet sizes. This increases the active surface area for the lipase enzyme and produces monoglycerides. Thus, the formation of smaller micelles gets increased, which helps to improve fat digestion. There is low efficacy of digesting fat in young chickens due to lack of lipase and bile salts activity (Tancharoenrat et al. 2014). However, lipase and bile supplementation have been shown to improve digestion and growth performance in broilers (Dersjant-Li and Peiskar 2005; Alzawqari et al. 2011). Thus, increasing the level of SSL supplementation reduced the FCR through increased
digestibility of fat and energy. In broilers, various emulsifiers such as 1,3 diacylglycerol and lysophospholipid suggested similar mechanisms (Zhao et al. 2015; Upadhaya et al. 2017; Liu et al. 2020). Serpunja and Kim (2019) demonstrated that emulsifiers supplemented to a low-energy diet can have comparable effects to a standard diet through improved fat digestibility in a study using SSL and Tween 20. However, DM digestibility was not improved in our study, which is similar to studies by Serpunja and Kim (2019) and Liu et al. (2020). Fat and energy digestibility changes were in such a linear trend that could not influence the DM digestibility. Interestingly, nutrient digestibility and growth performance did not show a clear improvement in the finishing broilers. At an early age, the incapability of fat digestion in broilers utilises exogenous emulsifier supplementation. With ageing, fat digestion capability in broilers gets increased where utilisation of exogenous emulsifier gets reduced. Tancharoenrat et al. (2013) reported that fat digestion ability in broilers keeps increasing until the 3rd week of age, and afterward it becomes equal. To understand the complete relationship between digestibility and growth performance, we would suggest testing nutrient digestibility at different ages, such as day 7 and day 21.

As Elmen et al. (2020) mentioned that SSL has some manipulating effect on specific bacterial groups in humans and Movagharnejad et al. (2020) found lysophospholipid to reduce the coliform bacterial population in broilers, we have conducted an excreta bacterial count on broilers receiving PC, NC and NC diets supplemented with a graded level of emulsifier. We did not find any significant changes in excreta microbial counts in broilers receiving SSL in energy reduced diets, which is similar to the results of Serpunja and Kim (2019).

In blood serum lipid parameters, only LDL was reduced with a linear tendency by SSL supplementation. As we have collected blood only at d 35, no significant difference in LDL, HDL, cholesterol, or triglycerides is understandable. Saleh et al. (2020); Upadhaya et al. (2018) and Liu et al. (2020) did not find any change in blood cholesterol, triglycerides, LDL, and HDL using emulsifiers at day 35. In addition, Roy et al. (2010) found no difference in LDL, HDL, and cholesterol on day 39. However, the same study found LDL and cholesterol were reduced in the blood by emulsifier supplementation on day 20. Zhao and Kim (2017) reported reduced LDL, and cholesterol in broilers at day 14 but a linear tendency of LDL reduction to day 28. From this discussion, we can conclude that the impact of SSL on the blood lipid parameters is highly age related.

5. Conclusion

In conclusion, sodium stearoyl-2-lactylate to energy reduced diets showed the capacity to enhance growth performance in broilers. Though the effect was not so prominent, SSL supplementation brought a gradual increase in energy and fat digestibility at early stages of broiler production.

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Ethical statement

This experiment methodology was approved (DK-1-2024) by Animal Care Committee, Dankook University, South Korea.

Disclosure statement

The authors declare no conflict of interest about this manuscript.

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