IMPACT OF USING DIFFERENT DIETARY LEVELS OF OIL WITH EMULSIFIER ON BROILERS PERFORMANCE, DIGESTIBILITY, AND LIPID PROFILE

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Abstract: This work was done to investigate the effect of dietary emulsifier supplementation (Lipidol) in different life stages of broilers (Ross 308) with different soybean oil levels with or without a reduced energy diet (-50 Kcal/Kg) on growth performance, digestibility, serum lipid profiles, carcass traits, and some minerals deposition in the whole body and tibia of broilers during a 42-day rearing period. A total of 240 one-day-old chicks were randomly assigned to sixteen experiment groups, three replicates of five birds each. The birds with a 4 × 2 factorial design received either four levels of soybean oil (1, 1.5, 2, and 2.5% of diet) or two levels of energy [recommended energy (control) or reduced energy (RE)]. The results described that dietary addition of an emulsifier to the control group significantly increased (P < 0.05) body weight (BW), body weight gain (BWG), decreased feed intake, and improve feed conversion ratio (FCR) in all periods except BWG and FCR during the finisher period, which showed non-significant difference. Moreover, the deposition of calcium and phosphorus in the whole body and tibia in all periods was significantly increased. The addition of an emulsifier significantly increased the dressing percent (P < 0.05) and abdominal fat percent (P < 0.0001), whereas there was non-significant difference among all groups in dressing %, intestine, gizzard, liver, and abdominal fat % by interaction, and all carcass traits were not affected by different levels of oil. Low-density lipoprotein (LDL) - cholesterol and total cholesterol concentrations also were decreased (P < 0.05) by emulsifier supplementation during all periods. Meanwhile, increased triglyceride levels were detected during starter and finisher periods. Fat and dry matter digestibility was increased (P < 0.05) by emulsifier supplementation in the group fed the lowest oil level. In conclusion, growth performance, digestibility, cholesterol, and bone quality were improved by dietary lipidol supplementation to reduced energy diets to a level that reached the control.

Key words: oil; emulsifier; energy; performance; minerals; broiler

Introduction

The poultry business is beneficial and gainful with great prospects of increasing the accessibility to high-quality protein in a short period. Poultry is considered the easiest and cheapest source of protein for human consumption (1). Recently, because of increasing energy costs, there is a major interest in maximizing the utilization of supplemental fats so, nutritionists try to elevate the dietary energy density to reach the nutrient requirements of high-performing poultry (2). Diets of poultry are usually required with excess nutrient and energy concentrations to meet the nutrient requirements of modern intensively reared birds. Fats and oils are added to achieve this high energy density in the diets (3). Energy is considered a major cost component factor in high-performance animal diets, such as broilers. Although reducing energy levels in broiler’s diets maintains the same performance that may be achieved by using emulsifiers, which improve fat digestibility and energy efficiency through facilitating the formation of emulsion droplets, which lowers the surface tension (4). The failure of young birds to
absorb fat efficiently was due to its poor emulsification ability and poor lipase activity. However, this problem improves with the age and adapts to deal with a higher level of unsaturated fatty acids (5). This explains that physiological functions necessary for the efficient digestion of fat are not properly developed in young broilers but for several weeks continually after hatching. This is because fat globules are not easily enzymatically digested and remain as indigestible residues within the intestinal tract, therefore an emulsion step is essential for the absorption of fat and many factors may affect supplemental fat utilization as the fat composition, physical form of the fat, and presence or absence of emulsifiers in diet (6). Also, the amount of dietary fat may affect the effectiveness of emulsifiers in the digestion of fat (7). It was hypothesized that the emulsifier added to broiler diets may improve the performance by the efficient utilization of energy and improving fat digestibility (8). Increasing levels of dietary fat interfered with mineral metabolism as it reduced the retention of calcium and magnesium by forming insoluble soaps, which were not absorbed (9). So, an emulsifier may overcome this problem. It was hypothesized that the exogenous emulsifier would facilitate the process of emulsification of fat to assist its efficient digestibility and absorption, so may help in preventing the formation of Ca soap and improving calcium absorption.

The primary objective of this study was to investigate the effectiveness of emulsifier supplementation in different life stages of the broiler on growth performance, carcass traits, serum lipid profile, digestibility, some minerals deposition in the whole body, and the tibia of broiler chickens to improve the performance and economic benefit by adding an emulsifier to reduced energy diets.

Material and methods

Ethical approval

The research was done following the institutional guidelines for the care and use of experimental animals approved by the Institutional Animal Care and use Committee, Zagazig University, Egypt (approval No. Zu- IACUC/2/F/48/2021).

Birds and management

Two hundred and forty, one-day-old chicks of a commercial meat-type (Ross308) were used for the experimental study. The chicks were 45 g initial body weight and randomly allocated to equal sixteen treatment groups, each containing three replicates, five chicks each, in a 4×2 factorial arrangement under a completely randomized design. Birds were reared in a naturally ventilated open house using a battery system with continuous lighting provided throughout the experiment. The starting temperature was 33°C then decreased gradually to 2°C each week until reach 21°C at the 6th week.

Experimental design and diets

For the experiment, iso-caloric and iso-nitrogenous broiler diets were formulated and offered in mash form. These diets had two energy levels, recommended energy (control) or reduced energy -50Kcal/Kg diet (RE) and four soybean oil levels 1, 1.5, 2, and 2.5% with fat emulsifier lipidol (positive lipidol) (PL) or without lipidol (negative lipidol) (NL) from Easy Bio, Inc company at a constant level (kg/ton). All contents were mixed by the mixer. The experiment lasted for 42 days and was divided into starter, grower, and finisher phases. The ingredients employed in feed formulation and calculated values of each experimental starter, grower, and finisher diet for basal or reduced energy diet are given in Tables 1 and 2, respectively.

The experimental diet was formulated to meet the nutrient requirements set by Ross 308 Broiler Nutrition Specification (2014). The utilization of two types of energy was to measure the efficiency of the emulsifier and the extent of its benefit when added to the reduced energy diet to improve the economic value. Also, we used four levels of soybean oil 1, 1.5, 2, and 2.5% to detect the best level that improves the palatability, resulting in improving the performance, and other parameters.

Procedures, sampling, and laboratory analysis

The broilers were weighed by pen, and feed intake was recorded at days 0, 10, 23, and 42, which was then used to calculate the body weight gain (BWG), feed intake, and feed conversion ratio (FCR).
Table 1: composition of the experimental diets (control diets with or without emulsifiers) /100 kg

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Feeding stages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starter stage</td>
</tr>
<tr>
<td>Soybean oil%</td>
<td>1%</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>58.14</td>
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<tr>
<td>Soybean meal</td>
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<td>Corn gluten, 60%</td>
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<td>Wheat bran</td>
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<tr>
<td>Mono calcium phosphate</td>
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<td>Calcium carbonate</td>
<td>1.72</td>
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<tr>
<td>L-lysin HCl 98%</td>
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</tr>
<tr>
<td>DL-Methionine, 99%</td>
<td>0.15</td>
</tr>
<tr>
<td>L-threonine 95.5%</td>
<td>0.12</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.25</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.1</td>
</tr>
<tr>
<td>Edible oil</td>
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</tr>
</tbody>
</table>

Mineral and vitamin premix: Each 1 kg diet contains vitamin A (12000 IU), vitamin D3 (5000 IU), vitamin E (80 IU), vitamin k3 (3.2 mg), vitamin B1 (3.2 mg), vitamin B2 (8.6 mg), vitamin B6 (4.3 mg), pantothenic acid (20 mg), vitamin B12 (0.017 mg), niacin (65 mg), folate (2.20 mg), biotin (0.22 mg), Fe (20 mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg). Emulsifier was added at a constant level (kg/ton).

Table 2: Composition of the reduced energy diets (-50 Kcal/ kg) (with or without emulsifiers)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Feeding stages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starter stage</td>
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<tr>
<td>Soybean oil%</td>
<td>1%</td>
</tr>
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<td>Yellow corn</td>
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<tr>
<td>Soybean meal</td>
<td>31.28</td>
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<tr>
<td>Corn gluten, 60%</td>
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<tr>
<td>Wheat bran</td>
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<tr>
<td>Mono calcium phosphate</td>
<td>1.75</td>
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<tr>
<td>Calcium carbonate</td>
<td>1.70</td>
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<tr>
<td>L-lysin HCl 98%</td>
<td>0.42</td>
</tr>
<tr>
<td>DL-Methionine, 99%</td>
<td>0.25</td>
</tr>
<tr>
<td>L-threonine 95.5%</td>
<td>0.12</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.7</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.6</td>
</tr>
<tr>
<td>Premix†</td>
<td>0.25</td>
</tr>
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</table>

Mineral and vitamin premix: Each 1 kg diet contains vitamin A (12000 IU), vitamin D3 (5000 IU), vitamin E (80 IU), vitamin k3 (3.2 mg), vitamin B1 (3.2 mg), vitamin B2 (8.6 mg), vitamin B6 (4.3 mg), pantothenic acid (20 mg), vitamin B12 (0.017 mg), niacin (65 mg), folate (2.20 mg), biotin (0.22 mg), Fe (20 mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg). Emulsifier was added at a constant level (kg/ton).

Also, blood samples were collected on days 10, 23, and 42 from the wing vein into a sterile syringe without anticoagulant; the obtained sera were stored at -20°C till used. The total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol in the serum samples were determined enzymatically according to the methods described previously (10-13). Very low-density lipoprotein (VLDL) cholesterol value can be calculated using the formula described by Bauer (14)

\[
\text{VLDL} = \frac{\text{triglycerides (mg/dL)}}{5}
\]

From day 32 to
broilers were fed diets mixed with titanium dioxide (0.5%) as an indigestible marker, which was analyzed using UV absorption spectrophotometry according to a previous protocol (15) to determine the apparent digestibility for dry matter and fat on the finisher stage. On day 10, 23, and 42, all plates under each cage were cleaned, and excreta samples were collected for 24 h, pooled within a cage, and then stored frozen at −20°C for the determination of fat digestibility. Excreta samples were dried at 60°C for 48 h, then allowed to cool at room temperature and milled (1mm screen) for fat determination (16). Fat digestibility at days 10 and 23 were determined by direct method and the apparent digestibility of DM on the finisher stage was calculated according to McDonald et al. (17) using the following formula:

\[
\text{Digestibility of dry matter} = \frac{\text{TiO}_2 \% \text{ in feces} - \text{TiO}_2 \% \text{ in diet}}{\text{TiO}_2 \% \text{ in feces}} \times 100
\]

Fat digestibility on the finisher stage was calculated according to Upadhaya et al. (18) using the following formula:

\[
\text{Ether extract digestibility} = [100 - \left((\frac{\text{TiO}_2 \% \text{ in diet}}{\text{TiO}_2 \% \text{ in feces}}) \times (\frac{\text{fat} \% \text{ in feces}}{\text{fat} \% \text{ in diet}})\right)] \times 100\%
\]

On day 42, three birds from each group were selected, fasted overnight, weighed then slaughtered by a sharp knife to complete bleeding, followed by plucking the feather, evisceration, and finally weighed to detect the dressing percentage. The dressed carcass weight, liver, gizzard, intestine, and abdominal fat were weighed and the right tibia was collected for ashing. A total of three birds per treatment at days 10 and 23 were euthanized by slaughter then burned in a muffle furnace at 600°C and Ash, Ca, and P were analyzed as stated previously (19) (methods 942.05; 927.02 and 965.17; respectively). Minerals were determined by the method described previously (20).

Statistical analysis

Data were analyzed as a completely randomized design using univariate analysis of the general linear model procedure of SAS software (SAS, 2016) and MSTAT-C statistical software. The model included the main effects of the emulsifier, energy, and different levels of oil as well as the interaction between the three factors. The level of significance was assessed at \( P < 0.05 \).

Results

Performance parameters

In the current study, the emulsifier significantly improved performance and decreased feed intake during all stages, but during the finisher period, there was no effect of emulsifier supplementation on BWG and FCR. The recommended energy (control) significantly increased BWT and BWG during all stages, also decreased the feed intake and FCR. 2.5% oil showed the highest BWT and BWG during the starter period, but 2% and 2.5% oil showed the highest BWT and BWG during grower, finisher, and overall periods. Feed intake was increased by higher levels of oil (2 and 2.5%), which also showed the lowest FCR during all stages.

By interaction, at day 23, the groups that received RE without emulsifier at oil 2.5% showed the highest feed intake followed by that received the emulsifier with the same treatment, which was followed by that received RE with the emulsifier at oil rate 2%. The latter was significantly more than that received 1.5% oil with the same treatment followed by the group that received control energy without emulsifier at oil 2%, which significantly increased the feed intake than that treated with the control energy with emulsifier at oil rate 2.5%. The latter was followed by the group treated with control energy without emulsifier at oil 1%, which significantly elevated feed intake than the group that received the emulsifier with the same treatment and also that received 1.5% oil, which showed the lowest feed intake. There was no significant difference between other groups in feed intake during the grower period.

During the finisher period, by interaction, the group that received control energy without emulsifier with 2.5% oil showed the highest BWG followed by that received RE with emulsifier with 1.5% oil and that received 1% oil with the same treatment of the latter showed the lowest BWG. There was no significant difference between other groups in BWG. Concerning the finisher feed intake, the groups that were treated by RE...
without emulsifier with 2% and 2.5% oil showed the highest feed intake followed by those that received 1% oil with the same treatment followed by those received 1% oil with RE with emulsifier supplementation. The latter significantly showed increasing in feed intake than that received 2.5% oil with control energy without emulsifier, which also significantly increased the feed intake more than that received 2% and 1.5% oil with the same treatment. The groups treated with 2.5% oil with control energy and emulsifier supplementation showed the lowest feed intake during the finisher period.

Concerning overall feed intake, by interaction, groups treated with 2 and 2.5% oil with RE without emulsifier showed the highest feed intake followed by 1 and 1.5% oil with the same treatment, which also followed by groups treated with 1 and 1.5% oil with RE with an emulsifier. The latter significantly increased overall feed intake than that received 2.5% oil with control energy and without emulsifier supplementation, which also significantly elevated the overall feed intake than those received 2 and 1.5% oil with the same treatment. The latter significantly increased overall feed intake than those treated with 2.5% oil with control energy with emulsifier supplementation, which was followed by the lowest level of overall feed intake that received 1% oil with the same treatment of the latter. Also, there was no significance among all groups in other parameters in all stages (Table 3, 4).

**Carcass quality traits**

Our present results showed that the addition of an emulsifier significantly increased dressing percent ($P < 0.05$) and abdominal fat percent ($P < 0.0001$). However, there was non-significance difference among all groups in dressing present, intestine, gizzard, liver, and abdominal fat percentages by interaction. All carcass traits are not affected by different oil levels (Table 5).

**Digestibility**

Our results showed that the addition of an emulsifier significantly increased fat digestibility in starter, grower, and finisher stages and increased dry matter digestibility at the end of the finisher stage. Also, the positive and negative energy did not affect the digestibility. The addition of oil at a rate of 1% and 1.5% significantly improved dry matter digestibility and fat digestibility by more than 2% and 2.5% oil in all periods of broilers. There was non-significance difference between all groups in fat and dry matter digestibility by interaction (Table 6).

**Total serum lipid**

Our results (Table 7) showed that during the starter period, total cholesterol and LDL-cholesterol significantly decreased with an emulsifier usage. Also, there is a significant increase in triglyceride and VLDL in groups that received emulsifiers with positive energy. Whereas there was non-significance difference in HDL in groups that received emulsifiers. The groups that received the oil at rates of 1 and 1.5% showed a significant increase in total cholesterol and LDL-cholesterol than others received oil at rates 2% and 2.5%. The latter showed a significant increase in triglyceride than groups that received oil at rates of 1% and 2%. There was non-significance among all groups in HDL and VLDL by interaction on days 10, 23, and 42.

By interaction during the grower period, groups that received emulsifiers showed significantly decreased total cholesterol, triglyceride, and LDL-cholesterol. Also, groups that received control energy showed a significant decrease in total cholesterol, while LDL-cholesterol and triglyceride significantly increased. Groups that received oil at a rate of 1.5% showed a significant decrease in total cholesterol and LDL-cholesterol, while groups that received the oil at a rate of 1% showed a significant increase in triglyceride and VLDL than other groups. There was a non-significance difference between all groups in HDL-cholesterol and VLDL by interaction.

By interaction during the finisher period, total cholesterol and LDL-cholesterol were significantly decreased and triglyceride was significantly increased in groups that received emulsifiers. Groups that received control energy showed a significant increase in total cholesterol and LDL-cholesterol as well as a significant decrease in triglyceride than the group that received RE. Groups that received 1.5% oil showed the lowest total cholesterol. The concentration of 2% and 2.5% oil significantly decreased total cholesterol and LDL-cholesterol than 1% oil.
Table 3: The effect of emulsifier supplementation on growth performance in broilers at days 10 and 23

<table>
<thead>
<tr>
<th>Addition of emulsifier (Lipidol)</th>
<th>Starter</th>
<th>Grower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BWT</td>
<td>BWG</td>
</tr>
<tr>
<td>Positive Lipidol (PL)</td>
<td>312</td>
<td>267</td>
</tr>
<tr>
<td>Negative Lipidol (NL)</td>
<td>306</td>
<td>261</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control energy (C)</td>
<td>311.8</td>
<td>266.8</td>
</tr>
<tr>
<td>Reduced energy(RE)</td>
<td>306.5</td>
<td>261.5</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>1.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Additions of Oil%

| 1     | 307b   | 262b   | 280b   | 1.11a  | 1119b  | 812b   | 1161b  | 1.43b  |
| 1.5   | 306b   | 261b   | 291b   | 1.12c  | 1135b  | 830b   | 1181b  | 1.41c  |
| 2     | 310a   | 265a   | 295a   | 1.11c  | 1191a  | 880a   | 1217a  | 1.39ab |
| 2.5   | 314a   | 269a   | 295a   | 1.09b  | 1217a  | 903a   | 1233a  | 1.36b  |
| P-Value | 0.001  | 0.001  | 0.04   | 0.04   | 0.0001 | 0.0001 | 0.0001 | 0.03   |
| SEM   | 1.03   | 1.03   | 1.2    | 0.007  | 8.4     | 8      | 9.2    | 0.02   |

Groups that received 1% and 1.5% oil showed a significant increase in triglyceride than those received 2% and 2.5% oil. There were non-significance differences among all groups in HDL-cholesterol and VLDL by interaction. By interaction, groups that received NE without emulsifier with oil 2.5% showed the highest level of cholesterol and LDL at days 10 and 23 and showed the lowest triglyceride with oil 1.5% at day 10. Reduced energy with emulsifier at oil 2.5% showed the lowest level of cholesterol and LDL at days 10 and 42 and also showed the highest triglyceride on days 10. On day 42, the control energy without emulsifier with 2.5% oil showed the highest level of cholesterol and LDL. Reduced energy with emulsifier with 2.5% oil showed the lowest level of cholesterol and LDL at day 42. Control energy with emulsifier at oil 2.5% and RE without emulsifier at 1.5% oil showed the lowest level of LDL at day 23. Reduced energy with emulsifier at 1% oil showed the highest level of triglyceride at days 23 and 42. The control energy with emulsifier at oil 2.5% showed the lowest level of triglyceride at day 23. There was no significance among all groups in HDL and VLDL in all periods.
Impact of using different dietary levels of oil with emulsifier on broilers performance, digestibility, ...

**Table 4**: The effect of emulsifier supplementation on growth performance in broilers at days 42 and overall

<table>
<thead>
<tr>
<th>Addition of emulsifier (Lipidol)</th>
<th>BWT</th>
<th>BWG</th>
<th>FI</th>
<th>FCR</th>
<th>BWT</th>
<th>BWG</th>
<th>FI</th>
<th>FCR</th>
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</thead>
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<tr>
<td>Positive Lipidol (PL)</td>
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<td>Negative Lipidol (NL)</td>
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<td>2916</td>
<td>4696</td>
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<td><em>P-Value</em></td>
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<td>SEM</td>
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<td>Control energy (C)</td>
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<td>1826</td>
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<tr>
<td>Additions of Oil%</td>
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<td></td>
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<td>2880b</td>
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<td>2</td>
<td>3035b</td>
<td>1845b</td>
<td>3187b</td>
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<td>2900b</td>
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<td>3007b</td>
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<td>0.006</td>
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<tr>
<td>SEM</td>
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<td>10.1</td>
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<td>13.4</td>
<td>13.4</td>
<td>16.5</td>
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</table>

**Interactions**

| 1                                | 2830| 1724bc| 3223bc | 1.87| 2830| 2785| 4749b| 1.70b|
| 1.5                              | 2889| 1824bcd| 3230b | 1.77| 2889| 2844| 4768b| 1.70b|
| 2                                | 2961| 1816bcd| 3243b | 1.79| 2961| 2916| 4814b| 1.67b|
| 2.5                              | 3010| 1811bcde| 3242b | 1.79| 3010| 2965| 4840b| 1.63b|
| 1                                | 2916| 1818bcde| 3123e | 1.72| 2916| 2871| 4525b| 1.57b|
| 1.5                              | 2908| 1730bcde| 3149bc| 1.82| 2908| 2863| 4601b| 1.60b|
| 2                                | 3073| 1867bc| 3143b | 1.68| 3073| 3028| 4611b| 1.50b|
| 2.5                              | 3100| 1893a| 3163b | 1.67| 3100| 3055| 4661a| 1.50b|
| 1                                | 2841| 1705a| 3203a | 1.88| 2841| 2796| 4701b| 1.67b|
| 1.5                              | 2904| 1767bde| 3215cd | 1.82| 2904| 2859| 4719b| 1.63b|
| 2                                | 3013| 1859bc| 3223bc | 1.74| 3013| 2968| 4768b| 1.60b|
| 2.5                              | 3040| 1830bcde| 3237b | 1.77| 3040| 2995| 4815b| 1.60b|
| 1                                | 2958| 1822bc| 3123c | 1.72| 2958| 2913| 4497b| 1.53b|
| 1.5                              | 2997| 1835bc| 3123c | 1.7| 2997| 2952| 4517b| 1.50b|
| 2                                | 3095| 1838bc| 3140c | 1.7| 3095| 3050| 4605b| 1.50b|
| 2.5                              | 3058| 1808bcde| 3103b | 1.72| 3058| 3013| 4541b| 1.50b|
| *P-Value*                        | 0.20| 0.03| 0.0001| 0.05| 0.20| 0.20| 0.0001| 0.30|
| SEM                              | 13.4| 10.1| 7.2  | 0.01| 13.4| 13.4 | 16.5 | 0.01|

NL: negative Lipidol; PL: positive Lipidol; RE: reduced energy; C: control energy; BWT: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SEM: standard error mean. Means with no common superscripts within the column of each classification are significantly different (*P < 0.05*)

**Levels of minerals in chick’s body and tibia**

Our results showed that Ca and P deposition significantly increased in all periods while Mn was significantly increased (*P < 0.0001*) at day 10 and decreased at days 23 and 42 by emulsifier supplementation. Control energy significantly improved Ca and P deposition in all periods but Mn significantly decreased with the control energy at days 10 and 23 and increased at day 42. Low oil levels (1%) significantly increased the deposition of Ca, P, and Mn.

By interaction, our results concerning Ca level showed that at days 10 and 23, groups received control energy without emulsifier by using oil at a rate of 1.5% showed the highest level of Ca deposited on the body followed by that received an emulsifier at oil 2% with the same energy level. The latter significantly elevated the level of Ca deposition more than the group received RE with an emulsifier at oil 1%, which also more than that treated by 2% oil with the same treatment. The latter significantly increased Ca deposition level more than that treated by oil at a
Table 5: The effect of emulsifier supplementation on carcass traits in broilers

<table>
<thead>
<tr>
<th>Addition of emulsifier (Lipidol)</th>
<th>Dressing %</th>
<th>Intestinal %</th>
<th>Gizzard %</th>
<th>Liver %</th>
<th>Abdominal fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Lipidol (PL)</td>
<td>79.21</td>
<td>6.39</td>
<td>3.50</td>
<td>2.76</td>
<td>0.67</td>
</tr>
<tr>
<td>Negative Lipidol (NL)</td>
<td>77.25</td>
<td>6.61</td>
<td>3.64</td>
<td>2.95</td>
<td>0.42</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.01</td>
<td>0.34</td>
<td>0.35</td>
<td>0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>0.72</td>
<td>0.222</td>
<td>0.138</td>
<td>0.153</td>
<td>0.024</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control energy (C)</td>
<td>78.38</td>
<td>6.39</td>
<td>3.52</td>
<td>2.87</td>
<td>0.54</td>
</tr>
<tr>
<td>Reduced energy (RE)</td>
<td>78.08</td>
<td>6.62</td>
<td>3.63</td>
<td>2.83</td>
<td>0.56</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.68</td>
<td>0.32</td>
<td>0.44</td>
<td>0.77</td>
<td>0.35</td>
</tr>
<tr>
<td>SEM</td>
<td>0.77</td>
<td>0.220</td>
<td>0.139</td>
<td>0.155</td>
<td>0.043</td>
</tr>
<tr>
<td>Additions of Oil %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>78.25</td>
<td>6.63</td>
<td>3.72</td>
<td>2.73</td>
<td>0.56</td>
</tr>
<tr>
<td>1.5</td>
<td>77.58</td>
<td>6.32</td>
<td>3.51</td>
<td>2.78</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>78.17</td>
<td>6.59</td>
<td>3.57</td>
<td>2.98</td>
<td>0.56</td>
</tr>
<tr>
<td>2.5</td>
<td>78.92</td>
<td>6.48</td>
<td>3.49</td>
<td>2.93</td>
<td>0.51</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.63</td>
<td>0.77</td>
<td>0.65</td>
<td>0.45</td>
<td>0.30</td>
</tr>
<tr>
<td>SEM</td>
<td>0.38</td>
<td>0.11</td>
<td>0.07</td>
<td>0.08</td>
<td>0.022</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL<em>RE</em>oil</td>
<td>77.33</td>
<td>7.03</td>
<td>3.87</td>
<td>3.57</td>
<td>0.37</td>
</tr>
<tr>
<td>1.5</td>
<td>76.33</td>
<td>6.73</td>
<td>3.60</td>
<td>2.70</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>75.25</td>
<td>7.03</td>
<td>3.57</td>
<td>3.03</td>
<td>0.40</td>
</tr>
<tr>
<td>2.5</td>
<td>76.00</td>
<td>6.47</td>
<td>3.70</td>
<td>3.10</td>
<td>0.42</td>
</tr>
<tr>
<td>NL<em>C</em>oil</td>
<td>78.33</td>
<td>6.33</td>
<td>3.57</td>
<td>2.70</td>
<td>0.48</td>
</tr>
<tr>
<td>1.5</td>
<td>76.30</td>
<td>6.82</td>
<td>3.68</td>
<td>3.10</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>78.33</td>
<td>5.87</td>
<td>3.07</td>
<td>2.30</td>
<td>0.43</td>
</tr>
<tr>
<td>PL<em>RE</em>oil</td>
<td>78.00</td>
<td>6.60</td>
<td>3.77</td>
<td>2.87</td>
<td>0.37</td>
</tr>
<tr>
<td>1.5</td>
<td>78.00</td>
<td>6.41</td>
<td>3.59</td>
<td>2.79</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>79.33</td>
<td>6.40</td>
<td>3.80</td>
<td>1.90</td>
<td>0.73</td>
</tr>
<tr>
<td>2.5</td>
<td>77.67</td>
<td>5.80</td>
<td>3.57</td>
<td>2.77</td>
<td>0.73</td>
</tr>
<tr>
<td>PL<em>C</em>oil</td>
<td>83.33</td>
<td>6.87</td>
<td>3.27</td>
<td>2.97</td>
<td>0.60</td>
</tr>
<tr>
<td>1.5</td>
<td>78.33</td>
<td>6.60</td>
<td>3.63</td>
<td>2.63</td>
<td>0.73</td>
</tr>
<tr>
<td>2</td>
<td>79.67</td>
<td>6.42</td>
<td>3.57</td>
<td>2.57</td>
<td>0.70</td>
</tr>
<tr>
<td>2.5</td>
<td>78.00</td>
<td>6.73</td>
<td>3.63</td>
<td>2.73</td>
<td>0.63</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.56</td>
<td>0.09</td>
<td>0.21</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>SEM</td>
<td>0.38</td>
<td>0.11</td>
<td>0.07</td>
<td>0.08</td>
<td>0.022</td>
</tr>
</tbody>
</table>

NL: negative Lipidol; PL: positive Lipidol; RE: reduced energy; C: control energy; SEM: standard error mean. Means with no common superscripts within the column of each classification are significantly different (P < 0.05).

The rate of 1% with control energy with an emulsifier, RE without emulsifier, and control energy without emulsifier respectively. Group treated by oil at a rate of 1.5 and 2.5% with control energy with emulsifier significantly increased Ca level more than that received RE with the same treatment. On days 10 and 23, the lowest Ca level showed in the group received RE without emulsifier at oil 2% and that treated with RE with emulsifier with 2.5% oil, respectively. Whereas, at day 42, the control energy without emulsifier with 1% oil showed the highest level of Ca followed by groups received RE or control energy with an emulsifier or at oil rate, 1.5%, which also significantly increased the Ca level more than with RE without emulsifier at 1% oil. The latter showed a significant increase in Ca level more than RE with or without emulsifier and control energy with or without emulsifier at oil 2% and control energy without emulsifier showed the lowest Ca level at oil 1.5%.

Concerning P, our results showed that at days 10 and 23, groups that received control energy without emulsifier using oil at a rate of 1.5% showed the highest level of P in the chick’s body followed by that received the control energy with
Table 6: The effect of emulsifier supplementation on digestibility in broilers

<table>
<thead>
<tr>
<th>Addition of emulsifier (Lipidol)</th>
<th>Starter Fat digestibility</th>
<th>Grower Fat digestibility</th>
<th>Finisher Fat digestibility</th>
<th>Dry matter digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Lipidol (PL)</td>
<td>83.15</td>
<td>86.56</td>
<td>91.88</td>
<td>90.41</td>
</tr>
<tr>
<td>Negative Lipidol (NL)</td>
<td>80.89</td>
<td>84.55</td>
<td>88.42</td>
<td>86</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.04</td>
<td>0.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>1.13</td>
<td>1.09</td>
<td>2.21</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Energy

| Control energy (C)              | 81.33                    | 84.87                    | 89.86                     | 88                      |
| Reduced energy (RE)             | 82.71                    | 86.25                    | 90.44                     | 88.41                   |
| P. Value                        | 0.2                      | 0.2                      | 0.5                       | 0.4                     |
| SEM                             | 1.16                     | 1.11                     | 2.27                      | 1.40                    |

Addition of Oil %

| 1                               | 84.67<sup>a</sup>        | 87<sup>a</sup>           | 95.37<sup>a</sup>         | 91.37<sup>a</sup>       |
| 1.5                             | 83.08<sup>b</sup>        | 87.54<sup>b</sup>        | 95.3<sup>b</sup>          | 91.3<sup>b</sup>        |
| 2                               | 81.85<sup>c</sup>        | 85.86<sup>c</sup>        | 90.36<sup>c</sup>         | 86.98<sup>c</sup>       |
| 2.5                             | 78.47<sup>d</sup>        | 81.82<sup>d</sup>        | 82.57<sup>d</sup>         | 83.19<sup>d</sup>       |
| P. Value                        | 0.002                    | 0.001                    | <0.0001                   | <0.0001                 |
| SEM                             | 0.58                     | 0.56                     | 1.12                      | 0.69                    |

Interactions

| 1                               | 83.81                    | 86.6                     | 94.41                     | 90.41                   |
| 1.5                             | 83.91                    | 88.09                    | 94.44                     | 90.45                   |
| 2                               | 81.24                    | 84.55                    | 84.9                      | 81.63                   |
| 2.5                             | 77.88                    | 81.7                     | 85.26                     | 83.38                   |
| 1                               | 82.87                    | 85.81                    | 93.72                     | 89.72                   |
| 1.5                             | 80.83                    | 85.91                    | 93.76                     | 89.76                   |
| 2                               | 79.87                    | 83.9                     | 83.24                     | 80.97                   |
| 2.5                             | 76.67                    | 79.86                    | 83.6                      | 81.72                   |
| 1                               | 86.03                    | 88.19                    | 96.86                     | 92.86                   |
| 1.5                             | 84.64                    | 88.81                    | 96.02                     | 92.02                   |
| 2                               | 83.86                    | 89.12                    | 96.91                     | 92.91                   |
| 2.5                             | 80.3                     | 82.91                    | 80.7                      | 83.66                   |
| 1                               | 85.97                    | 87.4                     | 96.47                     | 92.47                   |
| 1.5                             | 82.92                    | 87.36                    | 96.98                     | 92.98                   |
| 2                               | 82.44                    | 85.86                    | 96.4                      | 92.4                    |
| 2.5                             | 79.05                    | 82.83                    | 80.7                      | 84                     |
| P. Value                        | 1                        | 0.9                      | 1                         | 0.9                     |
| SEM                             | 0.58                     | 0.56                     | 1.12                      | 0.69                    |

NL: negative Lipidol; PL: positive Lipidol; RE: reduced energy; C: control energy; SEM: standard error mean. Means with no common superscripts within the column of each classification are significantly different (P < 0.05).

emulsifier by using oil at a rate of 2%, which significantly increased P deposition in the chick's body than groups that received RE with an emulsifier at oil 1%. The latter significantly increased P deposition more than that treated with the control energy with an emulsifier at oil 1%, which also deposited P more than groups treated with RE without an emulsifier at the same oil level 1%. The latter significantly elevated P deposition more than treating with control energy without an emulsifier at oil level 2%, which also increased P deposition more than groups treated with RE with an emulsifier supplementation with 1.5% oil, which also followed by RE without emulsifier with 1.5% oil, which also followed by groups treated with the same treatment but at the oil level 2% that showed the lowest level of P. However, at day 42, groups treated with the control energy with an emulsifier at the oil rate 1% showed the highest level of P deposition followed by the same treatment but with RE, the group treated with control energy at oil level 2.5% showed the lowest P level at day 42.
Table 7: The effect of emulsifier supplementation on serum lipid profile in broilers

<table>
<thead>
<tr>
<th>Addition of emulsifier (Lipidol)</th>
<th>Stater</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>TAG</td>
<td>HDL</td>
<td>LDL</td>
</tr>
<tr>
<td>Positive Lipidol (PL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Lipidol (NL)</td>
<td>148.7</td>
<td>45.6</td>
<td>49.3</td>
</tr>
<tr>
<td>P-value</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>7.77</td>
<td>3.46</td>
<td>1.06</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control energy (C)</td>
<td>127.5</td>
<td>54.9</td>
<td>51.3</td>
</tr>
<tr>
<td>Reduced energy (RE)</td>
<td>153.6</td>
<td>49.0</td>
<td>48.9</td>
</tr>
<tr>
<td>P-value</td>
<td>.0001</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td>SEM</td>
<td>7.17</td>
<td>5.84</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Concerning Mn, an emulsifier with RE using oil at a rate of 1% showed the highest level of Mn then groups received RE without an emulsifier with 1%oil and there was no significant difference between other groups in Mn at day 10 and there were no-significance differences between all groups in Mn at day 23 by interaction. But at day 42, the control energy without emulsifier with 1% oil showed the highest level of Mn which followed by RE without emulsifier with 2% oil that also significantly increased Mn level more than RE without an emulsifier with 2% oil that also significantly increased Mn level more than RE without an emulsifier with 1% oil that more than 1.5% oil the latter. More than 1 and 2.5% oil with RE with an emulsifier and control energy with an emulsifier with 2.5% oil and control energy with an emulsifier with 2.5% oil. Reduced energy and control energy with emulsifiers with 1.5% oil showed the lowest level of Mn (Table 8).

Discussion

Recommended dietary energy greatly affects the bird's growth performance during all life stages, which is in agreement with the study described previously (21) reporting that broilers fed basal diets had higher BWG (P < 0.05) than those received reduced energy diets during the first two weeks. Unlike Upadhaya et al. (8) who found that BW and BWG were not affected throughout the experiment by reducing dietary energy. The previous studies showed that the emulsifiers significantly improved birds' BW and BWG (21, 22), which is in the line with our current findings. Also, Tan et al. (23) reported that the BW of rice bran oil and emulsifier received group significantly increased in the 4th and 5th week. Supplementation of an emulsifier in incremental dose levels was expected to improve the live weight gain and feed conversion in broiler chickens according to the results of Roy et al. (24) who found that there was a 5% improvement in live weight in the group received an emulsifier at a rate of 1%
of dietary fat relative to the control group of birds and due to better utilization of dietary fat. Melegy et al. (25) found that the emulsifier based on lyssolecithin at level 0.25 or 0.5 kg/ton of feed significantly improves the performance.

Results of this study aren't in line with the findings of previous studies (26, 27) in which the addition of fat emulsifiers to broiler diets based on vegetable oil resulted in an increased weight gain during the finisher phase. However, no effect was found on starter weight gain that attributed to no activity of lipase in the starter phase but the weight gain increased in the finisher phase due to increased lipase activity leading to improved fat digestibility along with emulsification. It has been found that lysophosphatidylcholine utilization significantly increasing broilers body weight gain during the starter period (28).

In the current study, performance improvement was achieved by emulsifier supplementation in all periods except in BWG and FCR during the finisher period, with non-significant variation. This study supported the hypothesis that emulsifier supplementation would enhance the performance of broiler chickens by improving nutrient digestibility especially fat digestibility.
which is in the line with the research performed by Zampiga et al (29) who documented that the emulsifier was not significantly improved the final body weight and daily weight gain of broilers. Also, a previous study (30) found that emulsifiers added to a low energy density diet at a level of 0.05% with tallow as the fat source improved broiler's growth performance to the same levels as a high energy diet. As described previously (8), there was non-significance difference in BWG in broilers receiving low energy diet but supplementation of an emulsifier showed a linear elevate in BWG. Emulsifier (AVI-MUL TOP) supplementation on broiler diet at level 1 g/kg elevated body weight at days 12 and 22 and average daily gain from day 0 to 12, while decreased FCR from day 22 to 44 and from day 0 to 44 (31). Elevating the level of feed intake by reduced energy diet during all stages disagrees with another work (22) in which broilers fed the normal energy diet had higher feed intake (FI) in grower and overall period compared with that fed the reduced energy diet. Conversely, a previous study (32) demonstrated that a reduced energy diet (150 kcal/kg) decreased feed intake only during days 22 and 35. The emulsifier significantly decreased feed intake during all stages of broiler chicks, which isn't in the line with the findings of the study (23) who reported that feed intakes of birds that received rice bran oil with emulsifier significantly increased from the 2nd till the 4th week. Also, Lysophosphatidylcholine supplementation in broiler diet elevated average daily feed intake when used soy-fatty acids in the diets (33).

Our results are not in agreement with Kaczmarek et al. (34) and Zampiga et al. (29) who found that supplementation of emulsifiers did not affect FI of broiler chickens. Decreasing the FCR with emulsifier supplementation in our study was consistent with Kulkarni et al. (35), who found a beneficial effect by using the emulsifier on FCR and metabolizable energy. Contrary to our results, Lysophosphatidylcholine supplementation in broiler diet elevated FCR by 5.9% (33). In our study, high dietary oil levels showed the lowest FCR, which disagrees with the results of Abbas et al. (36) who reported that increased starter FCR with increasing fat level might be attributed to no lipase activity in the starter phase leading to lower absorption of fats (37). Halda and Ghosh (38) reported that low dietary energy improves the FCR by emulsifier supplementation as the emulsifier compensates for an energy reduction without decreasing growth rate. Upadhyay et al. (8) reported that the FCR was linearly reduced in low energy diet supplemented with different levels of emulsifier. In our results, the positive effects on growth performance were also due to an increase in apparent metabolizable energy and nutrition utilization in broilers through improving the digestion of soybean oil by emulsifier supplementation.

Increasing dressing (P < 0.05) and abdominal fat % by addition of emulsifiers was noticed in the present study as the emulsifier may increase the utilization of lipid for muscle formation and deposition of abdominal fat confirmed the findings of a previous study (24) in which fat increased in the whole body by approximately 50% in the emulsifier fed birds over that in the control group. Supplementation of an emulsifier probably facilitated the shunting of fats more towards body depot and reduced fat deposition in the liver (39). Previously, (40, 41) it was documented that the percent of abdominal fat was significantly increased by higher-level ME in the diet. Also, current results were in line with a previous finding (32) as there was non-significant variation in relative organ weight of broiler chickens between the control group and those received emulsifiers. In the present study, relative liver weight wasn't affected in the broiler-fed emulsifier contrary to Nagargoje et al. (42) who found that better weight of liver by adding soy lecithin into broilers diet as the liver is the principal place involved in lipids metabolism of the body, which accounts for 95% of the de novo fatty acid synthesis in birds (43).

Our findings supported the hypothesis that emulsifier supplementation would improve the broiler performance by increasing the digestibility of nutrients. Similarly, other researchers found that when a basal ration was supplemented with graded levels of an emulsifier such as lysophospholipid and 1, 3 diacylglycerols, fat digestibility of broilers and weaning pigs was increased (44, 45). Dietary emulsifier supplementation in the late growth phase of broiler has also been reported to enhance fat digestibility (46). Also, there is a linear increase (P < 0.05) in dry matter and fat digestibility in broilers fed different levels
of emulsifier (SSL and tween 20) (8). The present study is supported by the results obtained by previous studies (32, 36) in which the dry matter and ether extract digestibility significantly increased by emulsifier and low dietary fat. Fat emulsifiers act as an emulsifying agent for dietary fat and a stabilizer for other feed ingredients along with a higher degree of lipolysis of triglycerides leading to more micelle formation, digestion, and absorption of fats (32). Also, our results disagree with the previous studies (26, 47) which showed no effect of fat emulsifier supplementation at a low level in iso-caloric diets containing higher fat levels (5-6%) on digestibility that attributed to improper dietary emulsifier concentration leading to decrease uptake and utilization of dietary fat and other feed ingredients (48).

Total and LDL-cholesterol significantly decreased with emulsifier supplementation. Similarly, Roy et al. (24) showed that total cholesterol and LDL cholesterol reduced linearly (P < 0.05) at day 20 only and HDL cholesterol remained unaffected (P > 0.1). On the contrary, there was non-significant effect in the concentration of serum triacylglycerol (P > 0.1) by emulsifier, which caused the chylomicrons to be removed from the blood at a faster rate or delayed their release into the blood at a slower pace, so reducing the concentration of circulatory triacylglycerol. A previous study (49) reported that broilers received a higher metabolizable energy diet had higher levels of total cholesterol, triglyceride, and HDL.

Results of the present study aren’t fit with those of Upadhyaya et al. (8) who showed linear reduction (P = 0.051) in LDL-cholesterol in the serum of broilers fed emulsifier (SSL and tween 20). Previously, another study (31) reported that dietary supplementation of emulsifiers elevated total and HDL cholesterol (P = 0.02) plasma concentrations of broiler chicks. Also, it was previously documented that the addition of lysolecithin to the broiler diet did not affect serum total cholesterol, free fatty acid, or triglyceride concentrations (50). Also, Malapure and coauthors (51) demonstrated that Lysophospholipid significantly decreased total cholesterol on day 42 in contrast with GuerreiroNeto et al. (26) who reported that lipid profile levels of broiler chicken fed diets containing vegetable oil or animal fat were not affected by supplementation of emulsifier. Previously Cho et al. (32) found that broiler chickens received a diet with 0.05% emulsifier (sodium stearoyl-2-lactylate) had lower serum triglycerides than those who received high-energy diets without emulsifier. The exogenous emulsifier may act as a natural emulsifier and may play the role of cholesterol in fat emulsification and digestion so, the mechanism of reducing cholesterol by lipidol supplementation may be attributed to this reason. Also, the emulsifier helps to dissolve the free fatty acids, which are hardly soluble in bile salt micelle alone so improves the digestibility of saturated fatty acids and deposition of fatty acid in the body tissue (24).

The negative effects of reduced energy diets on bones in the present study showed the impairs of mineral deposition during all periods, but by emulsifier supplementation, the improving of Ca and P deposition has been observed and confirmed by Chen et al. (22) who indicated that lysophospholipid significantly improved the bone structure by increasing the Ca and P deposition in the tibia. In the present study, Ca, P, and Mn increased with low oil levels and positive energy in all stages but Mn significantly decreased with positive energy at days 10 and 23 and increased at day 42. On the contrary with our results, Leterrier et al. (52) found that bone development wasn’t affected by reduced energy diets. Another study (53) also found that Ca, P and ash percentages were increased in the tibia when birds received reduced energy diets. Unfortunately, as determined by several organizations 10-20 years ago, the mineral requirements of chickens may not support the optimal performance in today’s strain (broiler) (54). The results of the present study are in line with the findings of Wohl et al. (55) who found that a high-fat diet adversely affects intestinal Ca absorption and reduced the minerals in the bone of growing animals. The mechanism of the positive effects of emulsifier on bones of broilers can be described as emulsifier promoted the development of intestine by increasing absorption surface area and claudin-3 expression so, the emulsifier increased nutrients availability in the diets, which brought beneficial effects on bone quality (22).
Conclusion

Supplementing lipidol with 2 and 2.5% oil to reduced energy diets and control diets had positive effects on growth performance, and the latter could improve the dressing percent as well. Furthermore, dietary supplementation of lipidol as an emulsifier also showed positive effects on digestibility, and bone quality. Lipidol could become a potential feed additive for broilers to achieve better production.

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