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Impact of Using Emulsifier with Different Calcium and Soybean Oil Levels on Broilers Performance and Minerals Deposition

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Abstract

This study aimed to evaluate the effect of emulsifiers with different calcium and soybean oil levels on Ross 308 broiler performance, digestibility, carcass quality traits, and deposition of calcium, phosphorus, and manganese on the body and tibia of broilers. A total of 360 one-day-old chicks were randomly distributed into 24 treatment groups: three calcium to phosphorus ratios were used, 2:1, 1.8:1, and 1.6:1, and each ratio was divided into two positive control treatments with an emulsifier (Lipidol) and a negative control without an emulsifier; moreover, each treatment used four levels of soybean oil: 1%, 1.5%, 2%, and 2.5%. The results showed that emulsifier supplementation, low calcium levels (1.8:1 and 1.6:1), and high oil levels (2% and 2.5%) significantly elevated ($P<0.05$) body weight and body weight gain and reduced ($P<0.05$) feed intake and feed conversion ratio in all stages, except for body weight gain and feed conversion ratio during the finisher period, which showed a nonsignificant difference. Moreover, broilers fed the diet with emulsifier had higher dressing and abdominal fat percentage ($P<0.0001$) and those fed low Ca level (1.6:1) significantly ($P<0.0001$) showed the highest abdominal fat percentage. It can be concluded that an emulsifier with a low oil level could improve fat digestibility in broiler chickens. Furthermore, decreasing the level of calcium at a rate of 1.6:1 can improve fat digestibility and elevate ($P<0.0001$) the level of cholesterol on day 10; however, on days 23 and 42, cholesterol level was elevated by high calcium level (2:1). Emulsifier also elevates the cholesterol level at days 10 and 42. Broilers fed emulsifier, low oil level (1%), and high calcium levels at rates of 1.8:1 and 2:1 significantly ($P<0.05$) had elevating levels of calcium and phosphorus and decreased manganese levels on the broiler body and tibia.

KEYWORDS
Emulsifier, Performance, Calcium, Phosphorus, Digestibility

INTRODUCTION

Broilers are attractive for the production systems due to their high feed efficiency, short production cycle, and high biomass per unit of agricultural land. Thus, broiler meat plays an important role in food security for the rapidly increasing human population (Ansar *et al.*, 2004). Recently, there has been a major interest in maximizing the utilization of supplemental fats because of increasing energy costs; therefore, nutritionists are trying to elevate the dietary energy density to reach the nutrient requirements of high-performing poultry (Ravindran *et al.*, 2016). Lipids are triglycerides chemically composed of trimesters of glycerol and fatty acids. They are essential to protect the body against shock, maintain body temperature, and synthesize the hormone for muscular metabolism and the proper functioning of the central nervous system (Bjorntorp, 1991). Bile salts act as emulsifiers that divide fat into small droplets in an aqueous environment of the gastrointestinal tract. The emulsified fats are hydrolyzed by lipase enzyme and bile salts to form micelles. However, particularly in young birds, the digestive tract is not developed sufficiently to produce and secrete bile salts and lipase, so the absorption and digestion of high levels of dietary lipids are inefficient in young birds (Noy and Sklan, 1998; Al-Marzooqi and Leeson, 1999). However, this problem increases with age, and their bodies adapt to deal with a higher unsaturated fatty acids level (Meng *et al.*, 2004). Several previous studies have reported that exogenous emulsifiers increased the growth performance of broiler chickens because exogenous emulsifiers may be suitable alternatives for overcoming the problems associated with a high-fat diet and low bile acid excretion (Melegy *et al.*, 2010; Guerreiro Neto *et al.*, 2011; Zhang *et al.*, 2011). Calcium and phosphorus are important elements in poultry diet formulations (Li *et al.*, 2017) for bone formation and as enzyme cofactors (Li *et al.*, 2016) and for eggshell formation, blood coagulation, and nerve and muscle function (Klasing, 1998). Phosphorus is a key mediator of energy metabolism through ATP and an important element of nucleic acids, phospholipids, and eggshells. The concentration of Ca and P affect rates of intestinal absorption, endogenous intestinal losses, glomerular filtration, renal tubular reabsorption, bone accretion, and resorption (Li *et al.*, 2016).

Calcium imbalance is one of the problems responsible for economic losses in the poultry industry. Thus, maintaining a cal-

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cium and phosphorus ratio at 1:0.5 is fundamental for executing various functions in the body (Angel *et al*., 2005). Ca soaps are formed by the reaction of calcium with fat in the digestive tract and excreted (Whitehead *et al*., 1971; Whitehead and Fisher, 1975). Previous studies (Whitehead *et al*., 197l; Hakansson, 1975) have demonstrated that elevated levels of dietary fat interfered with mineral metabolism as fat reduced the retention of calcium and magnesium by forming insoluble soaps, which were not absorbed. These insoluble soaps from divalent cations and fatty acids influence both fatty acid metabolism and the availability of Ca. Compared with groups with Ca shortage, groups fed diets with appropriate calcium and phosphorus levels had better poultry performance (Kubena *et al*., 1974). The types and amounts of ingested dietary fats either improved or impaired the growth and development of bone and also modified bone mineral content in rats (Claassen *et al*., 1995) and chicks (Watkins *et al*., 1997). Calcium soaps formed from dietary unsaturated free fatty acids (oleic acid, C18:1) were absorbed by broilers as opposed to calcium soaps formed with the presence of saturated free fatty acids (palmitic acid, C16:0); thus, chicken-fed diets supplemented with palmitic acid compared with oleic acid showed reduction in bone ash and calcium content (Atteh and Leeson, 1984). Moreover, Corwin (2003) has reported that reducing bone density and increasing fracture risk in older and younger people may be due to high oil diets, especially those rich in saturated fatty acids.

Unfortunately, studies demonstrating the efficacy of emulsifiers with different Ca and oil levels in broiler diets are lacking. Therefore, this work attempted to evaluate the influence of using different levels of oil and calcium with or without emulsifiers on body growth performance parameters, digestibility, carcass traits, lipid profile, and minerals deposition in the body of broiler chickens.

MATERIALS AND METHODS

Ethical Approval

The research was conducted in accordance with 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

The experiment was carried out at Zagazig University, Egypt. A total of 360 one-day-old Ross 308 male broiler chicks were housed in a conventional house using a battery system. Upon arrival, they were weighed and randomly allocated equally to twenty-four treatment groups, each containing three replicates with five chicks for each one.

Experimental Design and Diets

For the experiment, isocaloric and isonitrogenous broiler diets were offered in mash form, fed according to the rearing phase (Tables 1, 2, and 3, respectively), and formulated to meet the nutrient requirements set by Ross 308 Broiler Nutrition Specification (2014). Three calcium into phosphorus ratios were used at rates of 2:1, 1.8:1, and 1.6:1. For each ratio, there were different soybean oil levels: 1%, 1.5%, 2%, and 2.5%. Each oil level was divided into a group without emulsifier supplementation or a group with an emulsifier (Lipidol) at a constant level (kg/ton)

Table 1. Composition of the experimental diets /100 kg with 2:1 Ca to P ratio (with or without emulsifiers).

Ingredients	Feeding stages											
	Starter stage				Grower stage				Finisher stage			
Soybean oil%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%
Yellow corn	58.14	57.1	55.61	54.27	63.39	62.38	61.2	60.07	69.67	68.58	67.28	66.47
Soybean meal	30.41	32.64	32.62	33.59	22.13	24.49	25.88	27.79	13.32	15.1	16.05	19.38
Corn gluten, 60%	5.6	4	4	3.3	9	7.35	6.4	5	11.7	10.45	9.8	7.5
Wheat bran	0.07	0.135	1	1.6	0.05	-	0.18	0.5	-	0.1	0.5	-
Monocalcium phosphate	1.75	1.75	1.75	1.75	1.53	1.52	1.52	1.51	1.45	1.45	1.45	1.4
Calcium carbonate	1.72	1.7	1.7	1.69	1.62	1.6	1.59	1.58	1.54	1.53	1.53	1.49
L-lysine HCl 98%	0.44	0.38	0.38	0.35	0.51	0.44	0.4	0.35	0.67	0.62	0.59	0.49
DL-Methionine, 99%	0.15	0.17	0.17	0.18	0.08	0.1	0.11	0.12	0.04	0.06	0.06	0.09
L-threonine 98.5%	0.12	0.12	0.12	0.11	0.11	0.1	0.09	0.09	0.13	0.12	0.12	0.11
Sodium bicarbonate	0.25	0.2	0.24	0.24	0.2	0.2	0.23	0.19	0.19	0.19	0.21	0.2
Common salt	0.1	0.1	0.2	0.2	0.18	0.12	0.2	0.1	0.1	0.1	0.2	0.2
Premix	0.25	0.2	0.21	0.22	0.2	0.2	0.2	0.2	0.19	0.2	0.21	0.2
Calculated composition												
ME(Kcal/kg)	3000	3000	3000	3000	3100	3100	3100	3100	3200	3200	3200	3200
CP	23	23	23	23	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5
EE	3.66	4.1	4.57	5.03	3.86	4.3	4.75	5.2	4.07	4.52	4.98	5.41
Ca	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.81	0.81	0.81	0.81
Available P	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43	0.4	0.4	0.4	0.4
Lysine	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19
Methionine	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51	0.48	0.48	0.48	0.48
Threonine	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.81	0.81	0.81	0.81

Vitamin and mineral premix: Each1kg diet contain vit. A (12000 IU), vit. D3 (5000 IU), vit. E (80 IU), vit. k3 (3.2mg), vit. B1 (3.2 mg), vit. B2 (8.6 mg), vit. B6 (4.3mg), pantothenic acid (20 mg),vit. B12 (0.017 mg), niacin (65 mg), folic acid (2.20 mg), biotin (0.22 mg), Fe (20mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg). Emulsifier added at a constant level Kg/Ton.

Table 2. Composition of the experimental diets /100 kg with 1.8:1 Ca to P ratio (with or without emulsifiers).

Ingredients	Feeding stages											
	Starter stage				Grower stage				Finisher stage			
Soybean oil%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%
Yellow corn	58.23	57.1	55.55	53.94	63.39	62.44	61.26	60.07	69.9	68.6	68.64	66.59
Soybean meal	31.15	32.63	32.64	32.51	22.13	24.9	26.38	27.77	14.62	15	15.75	20.1
Corn gluten, 60%	4.99	4	3.9	3.9	9	7	5.95	4.99	10.67	10.1	9.93	6.9
Wheat bran	0.2	0.2	1.4	2.7	0.05	0.07	0.4	0.6	-	0.2	0.2	-
Monocalcium phosphate	1.74	1.75	1.75	1.75	1.53	1.52	1.52	1.5	1.45	1.45	1.44	1.4
Calcium carbonate	1.44	1.41	1.41	1.41	1.33	1.33	1.32	1.3	1.27	1.27	1.27	1.23
L-lysine HCl 98%	0.42	0.38	0.38	0.38	0.51	0.43	0.39	0.35	0.63	0.6	0.6	0.47
DL-Methionine, 99%	0.15	0.17	0.17	0.17	0.08	0.1	0.11	0.12	0.05	0.06	0.06	0.1
L-threonine 98.5%	0.12	0.12	0.12	0.12	0.11	0.1	0.09	0.09	0.12	0.12	0.12	0.11
Sodium bicarbonate	0.16	0.4	0.25	0.22	0.47	0.3	0.2	0.26	0.1	0.16	0.19	0.2
Common salt	0.2	0.14	0.2	0.2	0.2	0.11	0.18	0.1	0.09	0.19	0.1	0.1
Premix	0.2	0.2	0.23	0.2	0.2	0.2	0.2	0.25	0.1	0.2	0.2	0.2
Calculated composition												
ME(Kcal/kg)	3000	3000	3000	3000	3100	3100	3100	3100	3200	3200	3200	3200
CP	23	23	23	23	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5
EE	3.65	4.1	4.58	5.05	3.86	4.3	4.75	5.2	4.07	4.52	4.52	5.41
Ca	0.86	0.86	0.86	0.86	0.77	0.77	0.77	0.77	0.72	0.72	0.72	0.72
Available P	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43	0.4	0.4	0.4	0.4
Lysine	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19
Methionine	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51	0.48	0.48	0.48	0.48
Threonine	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.81	0.81	0.81	0.81

Vitamin and mineral premix: Each1kg diet contain vit. A (12000 IU), vit. D3 (5000 IU), vit. E (80 IU), vit. k3 (3.2mg), vit. B1 (3.2 mg), vit. B2 (8.6 mg), vit. B6 (4.3mg), pantothenic acid (20 mg), vit. B12 (0.017 mg), niacin (65 mg), folic acid (2.20 mg), biotin (0.22 mg), Fe (20mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg). Emulsifier added at a constant level Kg/Ton.

Table 3. Composition of the experimental diets /100 kg with 1.6:1 Ca to P ratio (with or without emulsifiers).

Ingredients	Feeding stages											
	Starter stage				Grower stage				Finisher stage			
Soybean oil%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%	1%	1.50%	2%	2.50%
Yellow corn	58.17	57.04	55.42	53.91	63.36	62.43	61.23	60	69.91	68.61	68.64	66.63
Soybean meal	31.03	32.55	32.49	32.67	22.48	24.91	26.41	27.66	14.71	15.65	15.75	20.33
Corn gluten, 60%	4.99	4	3.9	3.7	8.6	6.94	5.85	4.99	10.6	10	9.93	6.7
Wheat bran	0.6	0.5	2	3	0.62	0.3	0.7	1	-	0.267	0.2	0.2
Monocalcium phosphate	1.74	1.75	1.75	1.75	1.53	1.52	1.52	1.5	1.44	1.45	1.44	1.4
Calcium carbonate	1.18	1.17	1.15	1.15	1.1	1.1	1.09	1.08	1.06	1.05	1.06	1
L-lysine Hcl 98%	0.42	0.38	0.38	0.38	0.5	0.43	0.39	0.35	0.63	0.6	0.6	0.46
DL-Methionine, 99%	0.15	0.17	0.17	0.17	0.08	0.1	0.11	0.12	0.05	0.06	0.06	0.1
L-threonine 98.5%	0.12	0.12	0.12	0.12	0.1	0.1	0.09	0.09	0.12	0.12	0.12	0.1
Sodium bicarbonate	0.2	0.4	0.22	0.25	0.23	0.28	0.21	0.26	0.09	0.25	0.25	0.2
Common salt	0.2	0.2	0.2	0.2	0.2	0.19	0.2	0.2	0.19	0..2	0.2	0.18
Premix	0.2	0.22	0.2	0.2	0.2	0.2	0.2	0.25	0.2	0.24	0.25	0.2
Calculated composition												
ME(Kcal/kg)	3000	3000	3000	3000	3100	3100	3100	3100	3200	3200	3200	3200
CP	23	23	23	23	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5
EE	3.66	4.11	4.59	5.06	3.87	4.3	4.76	5.21	4.07	4.52	4.52	5.41
Ca	0.77	0.77	0.77	0.77	0.69	0.69	0.69	0.69	0.64	0.64	0.64	0.64
Available P	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43	0.4	0.4	0.4	0.4
Lysine	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.19	1.19	1.19	1.19
Methionine	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51	0.48	0.48	0.48	0.48
Threonine	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.81	0.81	0.81	0.81

Vitamin and mineral premix: Each1kg diet contain vit. A (12000 IU), vit. D3 (5000 IU), vit. E (80 IU), vit.k3 (3.2mg), vit. B1 (3.2 mg), vit. B2 (8.6 mg), vit. B6 (4.3mg), pantothenic acid (20 mg),vit. B12 (0.017 mg), niacin (65 mg), folic acid (2.20 mg), biotin (0.22 mg), Fe (20mg), Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn (110 mg) Emulsifier added at a constant level Kg/Ton.

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Sampling, Procedures, and Laboratory Analysis

The following parameters were measured: body weight, body weight gain, feed intake and feed conversion ratio for the different stages of age starter, grower, and finisher. From days 32 to 42, broilers were fed diets mixed with titanium dioxide (0.5%) as an indigestible marker, which was analyzed using UV absorption spectrophotometry according to Myers *et al.* (2004) for determining the apparent digestibility of dry matter and fat. All plates were cleaned, and excreta samples were collected for 24 h, pooled within a cage, and then stored frozen at −20°C for evaluating fat digestibility during the starter and grower stages. Then, samples were dried at 60oC for 48 h and allowed to cool at room temperature, and milled (1 mm screen) for fat determination (method 954.02; AOAC, 2005). Fat digestibility during the starter and grower stages was determined using the direct method, and the apparent digestibility of dry matter during the finisher stage was calculated according to McDonald *et al.* (1981) using this formula:

digestibility of dry matter % = $\frac{\text{TiO2 \% feces} - \text{TiO2 \% diet}}{\text{TiO2 \% feces}} \times 100.$

In the finisher stage, ether extract digestibility was calculated according to Danicke *et al.* (1999) using this formula:

ether extract digestibility % $= [100 - \{(\frac{\text{TiO2 \% diet}}{\text{TiO2 \% feces}}) x (\frac{\text{fat \% feces}}{\text{fat \% diet}})\}] x 100$

Three birds per group were randomly selected at the end of each stage for blood samples, which were collected from the wing vein under aseptic conditions into a sterile syringe without anticoagulants and then centrifuged at 3,000 × g for 15 min. The sera were stored at −20oC until usage to determine the total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride values in the serum samples enzymatically according to the methods described by Meiattini *et al.* (1978); Grove (1979); Friedewald *et al.* (1972) and Bucolo and David (1973). Very-low-density lipoprotein (VLDL) cholesterol value can be calculated by the following formula: VLDL = triglycerides (mg/dL)/5, as described by Bauer (1982).

Dressing percentage, liver, gizzard, intestine, and abdominal fat were weighed at the end of the experimental period by selecting three birds from each group that fasted overnight, weighed, and then slaughtered by a sharp knife to complete bleeding. Subsequently, their feathers were plucked and evisceration was performed; then, the right tibia was collected for ashing. A total of three birds per treatment at days 10 and 23 were euthanized by slaughter and were burned in a muffle furnace at 600oC; then, Ca and P were analyzed according to the AOAC (1990) (methods 927.02 and 965.17, respectively). Minerals were determined by the method of Nation and Robinson (1971).

Statistical Analysis

All data were submitted to analyze the variance of the general linear model (GLM) procedure of SAS software (SAS, 2016) and MSTAT-C statistical software. Variability in the experiments' data was expressed as the standard error of means (SEM), and the level of $P < 0.05$ was considered significant.

RESULTS

Performance Parameters

The present results (Tables 4 and 5) showed that emulsifier supplementation significantly increased body weight (BW) and body weight gain (BWG) and significantly decreased the feed conversion ratio (FCR) and the feed intake during all periods. However, during the finisher period, there was no significance in BWG and FCR. Calcium (Ca) and phosphorus (P) utilization at a rate of 1.8:1 and 1.6:1 significantly showed the highest BW and BWG compared to a higher level of Ca at 2:1 in all periods. Moreover, the lowest Ca level significantly showed the lowest feed intake and FCR during all periods. High oil levels (2% and 2.5%) significantly improved BW, BWG, and FCR and significantly increased feed intake during all periods.

By interaction, during the starter period, BW and BWG were not significantly different among all groups; however, during the grower period, the group given a diet containing a Ca to P ratio of 1.8:1 with an emulsifier with 2.5% oil showed the highest BW and BWG, followed by the group given 2% oil with the same treatment. The group that received a diet containing a Ca to P ratio of 1.6:1 with an emulsifier with 2% oil showed a significant increase in BW and BWG compared to the groups given a Ca to P ratio of 2:1 with an emulsifier with 1.5% oil and Ca to P ratio of 2:1 without an emulsifier at 1% oil, respectively. Moreover, BW and BWG were not significantly different from the other groups during the grower period. By interaction, the group was given a diet containing a Ca to P ratio of 1.6:1 supplemented with an emulsifier with the highest oil level 2.5% showed the highest finisher BW, overall BW, and overall BWG, which were significantly more different than the group given the same treatment with 1.5% oil, followed by the group fed a diet with Ca to P ratio of 1.8:1 with emulsifier supplementation with 1% oil. The latter treatment significantly increased finisher BW, overall BW, and overall BWG more than the Ca to P ratio of 2:1 treatment with an emulsifier with 1% oil. Moreover, BW and BWG were not significantly different from other groups. Nevertheless, concerning the finisher BWG, the group treated with a 1.8:1 Ca to P ratio without an emulsifier with 2.5% oil showed the highest BWG, followed by that treated with a Ca to P ratio of 1.6:1 with an emulsifier with 1.5% oil. The latter treatment significantly improved BWG than the Ca to P ratio of 1.8:1 treatment without an emulsifier with 1% oil, followed by the Ca to P ratio of 2:1 with an emulsifier with 1.5% oil. Furthermore, there was no significant difference among the other groups in terms of the finisher BWG.

By interaction, during the starter period, the 1.8:1 Ca to P ratio with an emulsifier with 2.5% oil showed the highest feed intake, followed by the 1.6:1 Ca to P ratio with an emulsifier with 1% oil, and then the same treatment with 2% oil. The latter significantly increased feed intake more than the 1.6:1 Ca to P ratio without an emulsifier with 1% oil. Moreover, there was no significant difference among the other groups regarding feed intake. During the grower period, the group given a diet containing a Ca to P ratio of 1.6:1 with an emulsifier with 2% oil showed the highest feed intake, which was significantly more than that of the same treatment with 1.5% oil. The latter treatment showed significantly more feed intake than the 1.8:1 Ca to P treatment with an emulsifier with 1% oil. Moreover, there was no significant difference among the others in terms of feed intake.

By interaction, during the finisher stage, the group given a diet containing a Ca to P ratio of 2:1 with emulsifier supplementation with 2.5% oil showed the highest level of feed intake. The latter treatment significantly increased feed intake compared to the 1.6:1 Ca to P ratio with emulsifier supplementation with 2.5% oil. In this group, feed intake was significantly increased compared to the groups treated with a diet containing a Ca to P ratio of 2:1 with emulsifier supplementation with 2% oil and a Ca to P ratio of 1.8:1 with emulsifier supplementation with 1% oil.

Table 4. The effect of emulsifier supplementation with different calcium levels on performance in broilers at days 10 and 23.

		Starter				Grower			
		BWT (g)	BWG (g)	FI (g)	FCR	BWT (g)	BWG (g)	FI (g)	FCR
Addition of Emulsifier									
With Lipidol (PL)		311	266	283	1.07	1210	899	1200	1.34
Without Lipidol (NL)		308	263	288	1.1	1190	882	1217	1.38
P-Value		0.001	0.001	0.001	0.000	0.002	0.009	0.001	0.001
SEM		2.4	2.4	2.2	0.01	9.7	8.5	8.8	0.01
Calcium levels (Ca: P)									
2:01		305^{b}	260^{b}	289^{a}	1.12^{a}	1189^{b}	884^{b}	1223^{a}	1.4^{a}
1.8:1		312^{a}	267^{a}	287^{a}	1.07^{b}	1198^{a}	886^{b}	1204^{ab}	1.36^{ab}
1.6:1		311^{a}	266^{a}	281^{b}	1.06^{b}	1213^{a}	902^{a}	1199^{b}	1.33^{b}
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	0.009	0.04	0.001	0.002
SEM		1.2	1.2	1.14	0.006	4.9	4.3	4.5	0.008
Addition of oil%									
1		301^{b}	256^{b}	281^{b}	1.1^{a}	1171^{b}	870^{b}	1192^{b}	1.4^{a}
1.5		302^{b}	257^{b}	283^{b}	1.1^{a}	1177^{b}	875^{b}	1208^{ab}	1.4^{a}
2		315^{a}	270^{a}	287^{ab}	1.06^{b}	1217^{a}	902^{a}	1214^{ab}	1.36^{b}
2.5		319^{a}	274^{a}	292^{a}	1.07^{b}	1236^{a}	917^{a}	1221^{a}	1.33^{b}
P-Value		0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004
SEM		1.2	1.2	1.14	0.006	4.9	4.3	4.5	0.008
Interactions									
NL*Ca: P (2:1) *oil	1	287	242	287^{bcdef}	1.19^{a}	1140^{h}	854^{fgh}	1210^{abcd}	1.43
	1.5	291	246	293^{abc}	1.19^{a}	1173^{fgh}	882^{cdefgh}	1235^{ab}	1.4
	2	308	263	297^{ab}	1.13^{bc}	1187^{defgh}	879^{cdefgh}	1210^{abcd}	1.4
	2.5	319	274	291^{bcd}	1.06^{defg}	1219^{cdef}	900^{cdef}	1225^{abc}	1.37
PL*Ca: P (2:1) *oil	1	303	258	285^{bcdef}	1.1^{cde}	1169^{fgh}	865^{efgh}	1213^{abcd}	1.4
	1.5	291	246	285^{bcdef}	1.16^{ab}	1174^{fgh}	883^{cdefgh}	1214^{abcd}	1.33
	2	317	272	287^{bcdef}	1.05^{defg}	1233^{abcd}	916^{abcde}	1240^{a}	1.37
	2.5	320	275	287^{bcdef}	1.04^{efg}	1215^{cdef}	894^{cdef}	1238^{ab}	1.4
NL*Ca :P (1.8:1)*oil	1	304	259	285^{bcdef}	1.1^{cde}	1145^{gh}	842^{gh}	1212^{abcd}	1.4
	1.5	307	262	280^{defg}	1.07^{def}	1142^{h}	835^{h}	1245^{a}	1.5
	2	315	270	280^{defg}	1.04^{efg}	1232^{abcd}	917^{abcd}	1243^{a}	1.37
	2.5	317	272	297^{ab}	1.09^{cde}	1185^{defgh}	868^{defgh}	1223^{abc}	1.4
PL*Ca: P (1.8:1)*oil	1	309	264	279^{defg}	1.06^{defg}	1179^{efgh}	870^{defgh}	1156^{fgh}	1.33
	1.5	309	264	279^{defg}	1.06^{defg}	1196^{cdefg}	887^{cdefg}	1127^{h}	1.27
	2	317	272	289^{bcde}	1.06^{defg}	1228^{bcde}	910^{bcde}	1175^{def}	1.3
	2.5	317	272	303^{a}	1.11^{bcd}	1279^{a}	962^{a}	1246^{a}	1.3
NL*Ca :P (1.6:1)*oil	1	302	257	276^{fg}	1.07^{def}	1205^{cdef}	902^{cdef}	1225^{abc}	1.37
	1.5	308	263	282^{cdefg}	1.07^{def}	1186^{defgh}	878^{cdefgh}	1230^{ab}	1.37
	2	314	269	295^{ab}	1.1^{cde}	1194^{defg}	880^{cdefgh}	1167^{efg}	1.33
	2.5	320	275	293^{abc}	1.07^{def}	1272^{ab}	952^{ab}	1184^{cdef}	1.23
PL*Ca: P(1.6:1)*oil	1	302	257	271^{g}	1.06^{defg}	1187^{defgh}	885^{cdefgh}	1137^{gh}	1.3
	1.5	305	260	278^{efg}	1.07^{def}	1188^{defgh}	883^{cdefgh}	1198^{bcde}	1.37
	2	318	273	272^{g}	1^{g}	1227^{bcde}	909^{bcde}	1247^{a}	1.4
	2.5	320	275	278^{efg}	1.01^{fg}	1245^{abc}	924^{abc}	1208^{abcd}	1.3
P-Value		0.17	0.17	0.04	0.02	0.01	0.01	<0.0001	0.1
SEM		1.2	1.2	1.14	0.006	4.9	4.3	4.5	0.008

NL: negative lipidol; PL: positive lipidol; Ca:P: calcium into phosphorus ratio; g: gram; SEM: standard error mean; a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

Table 5. The effect of emulsifier supplementation with different calcium levels on performance in broilers at day 42 and overall.

		Finisher				Overall			
		BWT (g)	BWG (g)	FI (g)	FCR	BWT (g)	BWG (g)	FI (g)	FCR
Addition of Emulsifier									
With Lipidol (PL)		3020	1810	3176	1.76	3020	2975	4658	1.56
Without Lipidol (NL)		2994	1804	3198	1.77	2994	2949	4703	1.6
P-Value		0.004	0.5	0.001	0.3	0.004	0.004	0.000	0.000
SEM		22.7	17.5	13.6	0.02	22.7	22.7	19.08	0.01
Calcium levels (Ca: P)									
2:01		2956^{b}	1768^{b}	3216^{a}	1.82^{a}	2956^{b}	2911^{b}	4727^{a}	1.63^{a}
1.8:1		3021^{a}	1823^{a}	3186^{ab}	1.75^{b}	3021^{a}	2976^{a}	4676^{b}	1.57^{b}
1.6:1		3044^{a}	1831^{a}	3159^{b}	1.73^{b}	3044^{a}	2999^{a}	4639^{b}	1.53^{c}
P-Value		<0.0001	<0.0001	<0.0001	0.000	<0.0001	<0.0001	<0.0001	<0.0001
SEM		11.4	8.7	6.9	0.009	11.4	11.4	9.8	0.008
Addition of oil%									
1		2911^{c}	1741^{b}	3143^{c}	1.81^{a}	2911^{c}	2866^{c}	4616^{c}	1.62^{a}
1.5		2951^{b}	1774^{b}	3179^{b}	1.8^{a}	2951^{b}	2906^{b}	4670^{b}	1.61^{a}
2		3068^{a}	1851^{a}	3199^{ab}	1.73^{b}	3068^{a}	3023^{a}	4699^{ab}	1.55^{b}
2.5		3098^{a}	1862^{a}	3225^{a}	1.73^{b}	3098^{a}	3053^{a}	4737^{a}	1.54^{b}
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SEM		11.4	8.7	6.9	0.009	11.4	11.4	9.8	0.008
Interactions									
NL*Ca : P (2:1)*oil	1	2828^{j}	1688^{i}	3203^{defg}	1.9	2828^{j}	2783^{j}	4699^{defg}	1.7
	1.5	2909^{fghi}	1736^{hi}	3187^{efgh}	1.83	2909^{fghi}	2864^{fghi}	4714^{cdef}	1.63
	2	2985de	1798^{defgh}	3250^{abcd}	1.8	2985de	2940de	4757^{abcd}	1.63
	2.5	3010^{cd}	1791^{defgh}	3260^{abc}	1.83	3010^{cd}	2965^{cd}	4775^{abc}	1.6
PL*Ca : P (2:1)*oil	1	2852^{ij}	1683^{i}	3197^{efg}	1.9	2852^{ij}	2807^{ij}	4695^{defg}	1.7
	1.5	2868^{hij}	1694^{i}	3198^{efg}	1.9	2868^{hij}	2823^{hij}	4697^{defg}	1.67
	2	3063^{abc}	1830^{bcdef}	3156^{ghi}	1.7	3063^{abc}	3018^{abc}	4682^{fg}	1.57
	2.5	3133^{a}	1919^{a}	3274^{a}	1.7	3133^{a}	3088^{a}	4799^{a}	1.57
NL*Ca :P(1.8:1)*oil	1	2905^{ghi}	1760^{fgh}	3200^{efg}	1.83	2905^{ghi}	2860^{ghi}	4697^{defg}	1.63
	1.5	2960^{defg}	1818^{cdefg}	3200^{efg}	1.77	2960^{defg}	2915^{defg}	4725^{bcdef}	1.6
	2	3089^{ab}	1858^{abcd}	3230^{abcde}	1.73	3089^{ab}	3044^{ab}	4753^{abcde}	1.6
	2.5	3108^{a}	1923^{a}	3262^{ab}	1.7	3108^{a}	3063^{a}	4782^{ab}	1.6
PL*Ca : P(1.8:1)*oil	1	2928^{efgh}	1749^{ghi}	3065^{j}	1.77	2928^{efgh}	2883^{efgh}	4500^{i}	1.57
	1.5	2966^{defg}	1770^{efgh}	3119^{i}	1.8	2966^{defg}	2921^{defg}	4525^{hi}	1.57
	2	3097^{a}	1869^{abc}	3232^{abcde}	1.73	3097^{a}	3052^{a}	4696^{defg}	1.5
	2.5	3113^{a}	1835^{bcde}	3176^{fgh}	1.7	3113^{a}	3068^{a}	4725^{bcdef}	1.5
NL*Ca :P(1.6:1)*oil	1	2978de	1774^{efgh}	3074^{j}	1.7	2978de	2933de	4575^{h}	1.57
	1.5	2973^{def}	1787^{efgh}	3159^{ghi}	1.73	2973^{def}	2928^{def}	4671^{fg}	1.6
	2	3086^{ab}	1892^{ab}	3184^{efgh}	1.7	3086^{ab}	3041^{ab}	4646^{g}	1.5
	2.5	3097^{a}	1825^{bcdef}	3164^{fghi}	1.73	3097^{a}	3052^{a}	4642^{g}	1.5
PL*Ca : P(1.6:1)*oil	1	2977de	1790^{defgh}	3120^{i}	1.77	2977de	2932de	4528^{hi}	1.53
	1.5	3028^{bcd}	1840^{bcde}	3212^{cdef}	1.77	3028^{bcd}	2983^{bcd}	4688^{efg}	1.57
	2	3087^{ab}	1860^{abcd}	3144^{hi}	1.7	3087^{ab}	3042^{ab}	4663^{fg}	1.5
	2.5	3126^{a}	1882^{abc}	3214^{bcdef}	1.7	3126^{a}	3081^{a}	4701^{defg}	1.5
P-Value		0.02	0.001	0.000	0.08	0.02	0.02	0.007	0.3
SEM		11.4	8.7	6.9	0.009	11.4	11.4	9.8	0.008

NL: negative lipidol; PL: positive lipidol; Ca:P: calcium into phosphorus ratio; g: gram; SEM: standard error mean. a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

By interaction, during the overall period, the group given a 2:1 Ca to P treatment with emulsifier supplementation with 2.5% oil showed the highest overall feed intake. This treatment significantly increased feed intake more than the 1.8:1 Ca to P treatment with emulsifier supplementation with 2.5% oil. The latter treatment increased feed intake more than the 1.6:1 Ca to P treatment without emulsifier supplementation with 2% oil, which also showed more feed intake than the 1.6:1 Ca to P treatment with emulsifier supplementation with 1% oil.

By interaction, during the starter period, the utilization of the 2:1 Ca to P treatment without an emulsifier with 1% and 1.5% oil levels resulted in the highest FCR, followed by the same treatment with 2% oil. The latter treatment was followed by the 1.6:1 Ca to P treatment without an emulsifier with 1%, 1.5%, and 2.5% oil levels. The group given the 1.6:1 Ca to P treatment with an emulsifier with 2% oil showed the lowest FCR. Furthermore, there was no significant difference among all groups in FCR during all periods by interaction.

Carcass Quality Traits

Supplementation of emulsifiers significantly increased dressing percentage and abdominal fat; however, the intestinal, liver and gizzard percentages were not significantly different among all groups by emulsifier supplementation, Ca, and oil levels. Moreover, the 1.6:1 Ca:P ratio showed the highest abdominal fat percentage, whereas the 2:1 Ca:P ratio showed the lowest abdominal fat percentage. However, by interaction, there were no significant differences between all groups in dressing, intestinal, liver, and gizzard percentages (Table 6).

Digestibility

Emulsifier significantly improved fat digestibility during the starter, grower, and finisher stages and improved dry matter (DM) digestibility during the finisher phase. During the starter period, the most efficient level of Ca that improved fat digestibility was the lowest level of Ca 1.6:1, and Ca to P ratio of 1.8:1 during the finisher period. Ca to P ratio of 2:1 significantly improved DM digestibility more than the 1.6:1 ratio. Moreover, there was no significant difference between the Ca:P ratios of 2:1 and 1.8:1 in DM digestibility during the finisher period. By interaction, fat digestibility was not significantly different among all groups during all periods (Table 7).

Serum Lipid Profile

During the starter period, emulsifier supplementation significantly increased cholesterol and decreased HDL and LDL cholesterol. Moreover, emulsifier supplementation did not significantly affect the triacylglycerol (TAG) and VLDL values. The 1.6:1 Ca to P treatment showed the highest levels of cholesterol, LDL, TAG, and VLDL; the Ca level did not significantly affect HDL. Furthermore, the 2% oil level showed the highest cholesterol and TAG. The 1% oil level resulted in the highest level of LDL. VLDL was not significantly different in terms of different oil levels (Table 8).

During the grower period, emulsifier supplementation significantly decreased cholesterol and LDL. Emulsifier supplementation did not significantly affect TAG, HDL, and VLDL. The 2:1 Ca to P ratio showed the highest level of cholesterol, LDL, and TAG. The 2% oil level resulted in the highest cholesterol and LDL. The 1% oil level resulted in the highest level of TAG. VLDL was not significantly different among groups with different oil levels.

During the finisher period, emulsifier supplementation significantly increased total cholesterol and LDL and decreased TAG. Moreover, Results showed that HDL and VLDL were not significantly different between groups treated with an emulsifier. The 2.5% and 1% oil levels significantly showed the highest cholesterol and LDL. HDL and VLDL were not significantly different among groups treated with different oil levels.

By interaction, during the starter period, the group given a 1.8:1 Ca to P treatment without emulsifier supplementation with 2% oil showed the highest cholesterol level, followed by the group given a 2:1 Ca to P treatment with emulsifier supplementation with 1.5% oil. The group given a 2:1 Ca to P treatment with emulsifier supplementation with 2.5% oil showed the lowest cholesterol level.

Concerning TAG, birds given a 1.6:1 Ca to P treatment with emulsifier supplementation with 2.5% oil showed the highest TAG, which was more than the group given the same treatment with 2% oil. Moreover, TAG was not significantly different between the latter group and the group treated with a diet containing a 1.8:1 Ca to P ratio with emulsifier supplementation with 1.5% oil. Furthermore, the group given a 1.8:1 Ca to P treatment without emulsifier supplementation with 1.5% oil showed the lowest TAG level.

Regarding HDL, the groups that received a 2:1 Ca to P treatment with emulsifier supplementation with 1% oil level and a 1.8:1 Ca to P treatment without emulsifier supplementation with 1.5% oil showed the highest HDL levels. However, the group given a 1.6:1 Ca to P treatment without emulsifier supplementation with 2.5% oil showed the lowest HDL.

Regarding LDL, utilization of a 1.6:1 Ca to P ratio with emulsifier supplementation with 1% oil showed the highest LDL. However, the group given a 1.8:1 Ca to P treatment without emulsifier supplementation with 1.5% oil showed the lowest LDL. Concerning VLDL, the 1.6:1 Ca to P treatment with emulsifier supplementation with 2.5% oil showed the highest level of VLDL, followed by the treatment with the same Ca level without emulsifier supplementation and 1.5% oil. Furthermore, utilization of the 1.8:1 Ca to P with emulsifier supplementation with 1% and 2% oil levels showed the lowest VLDL. VLDL was not significantly different from the others.

During the grower period, by interaction, there was no significant difference between the group given a 2:1 Ca to P ratio without emulsifier supplementation with 2% oil and the group was given the same level of Ca with emulsifier supplementation with 1% oil. The latter group showed the highest cholesterol level, followed by the group treated with a 1.6:1 Ca to P diet with emulsifier supplementation with 1% oil. The latter group also showed a significantly elevated cholesterol level compared to the group treated with a 1.8:1 Ca to P diet with emulsifier supplementation with 1.5% oil, which was more significant than that in the group given the same Ca level without emulsifier at 2.5% oil. Utilization of a 1.8:1 Ca to P treatment with an emulsifier with 2% oil significantly elevated cholesterol compared to the same treatment with a 2.5% oil level, which showed the lowest level of cholesterol. During the grower period, there was no significant difference among the others in cholesterol.

Concerning TAG, the group given a 1.8:1 Ca to P treatment with emulsifier supplementation with 1% oil showed the highest TAG level, which was more significant than in the group treated with a 1.6:1 Ca to P ratio with the same other treatment. The latter group had a more TAG level than the group treated with a 1.8:1 Ca to P ratio without an emulsifier with 2% oil. The TAG level was more significant in the latter group than that in the group treated with a 1.6:1 Ca to P ratio without an emulsifier with 1.5% oil.

Table 6. The effect of emulsifier supplementation with different calcium levels on carcass traits in broilers.

		Dressing %	Intestinal%	Gizzard%	Liver%	Fat%
Addition of Emulsifier						
With Lipidol (PL)		81	6.05	3.53	2.88	0.7
Without Lipidol (NL)		78	6	3.69	2.76	0.5
P-Value		<0.0001	0.8	0.3	0.3	<0.0001
SEM		0.63	0.22	0.14	0.13	0.02
Calcium levels (Ca: P)						
2:01		79	6.39	3.52	2.87	0.5^{b}
1.8:1		79	6.01	3.57	2.65	0.6^{ab}
1.6:1		80	5.69	3.75	2.95	0.7^{a}
P-Value		0.3	0.03	0.4	0.1	<0.0001
SEM		0.3	0.1	0.07	0.06	0.02
Addition of oil%						
1		79	5.98	3.51	2.97	0.6
1.5		79	6.29	3.69	2.85	0.6
2		80	5.87	3.62	2.82	0.6
2.5		80	5.97	3.63	2.66	0.6
P-Value		0.3	0.5	0.8	0.4	0.4
SEM		0.3	0.1	0.07	0.06	0.02
Interactions						
NL*Ca: P (2:1)*oil	1	78	6.33	3.57	2.7	0.5
	1.5	78	5.87	3.07	2.3	0.4
	2	78	6.83	3.97	3.3	0.4
	2.5	77	6.6	3.77	2.87	0.4
PL*Ca: P (2:1)*oil	1	80	6.73	3.63	2.73	0.6
	1.5	80	6.87	3.8	3.33	0.7
	2	82	5.9	3.1	2.93	0.7
	2.5	81	5.97	3.23	2.8	0.6
NL*Ca :P (1.8:1)*oil	1	76	6.8	3.47	3.07	0.5
	1.5	74	6.1	3.9	2.57	0.4
	2	81	5.13	3.7	2.3	0.5
	2.5	79	6.47	3.43	2.2	0.5
PL*Ca: P(1.8:1)*oil	1	79	5.73	3.13	3.03	0.7
	1.5	81	6.07	3.5	2.83	0.7
	2	80	6.33	3.3	2.9	0.7
	2.5	82	5.43	4.1	2.27	0.7
NL*Ca :P (1.6:1)*oil	1	79	4.6	3.77	3.1	0.6
	1.5	82	6.1	4.1	3.2	0.5
	2	79	5.5	4	2.6	0.5
	2.5	78	5.7	3.5	2.97	0.5
PL*Ca : P(1.6:1)*oil	1	80	5.67	3.47	3.17	0.7
	1.5	79	6.73	3.77	2.87	0.7
	2	82	5.53	3.63	2.87	0.8
	2.5	82	5.67	3.73	2.87	0.7
P-Value		0.06	0.2	0.4	0.4	0.9
SEM		0.3	0.1	0.07	0.06	0.02

NL: negative Lipidol; PL: positive Lipidol; Ca:P: calcium into phosphorus ratio; SEM: standard error mean. a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

Table 7. The effect of emulsifier supplementation with different calcium levels on digestibility in broilers.

		Starter	Grower	Finisher	
		Fat digestibility %	Fat digestibility %	Fat digestibility %	DM digestibility %
Addition of Emulsifier					
With Lipidol (PL)		82	87	92	94
Without Lipidol (NL)		80	85	88	89
P-Value		0.01	0.03	<0.0001	<0.0001
SEM		0.94	0.83	1.5	1.05
Calcium levels (Ca: P)					
2:01		79^{b}	87	89^{b}	92^{a}
1.8:1		80^{b}	86	93^{a}	91^{ab}
1.6:1		83^{a}	85	87^{bc}	90^{b}
P-Value		<0.0001	0.3	<0.0001	0.03
SEM		0.48	0.43	0.6	0.6
Addition of oil%					
1		82^{a}	88^{a}	91.7^{a}	94^{a}
1.5		82^{a}	87^{a}	92^{a}	94^{a}
2		81^{a}	86^{a}	88^{b}	90^{b}
2.5		78^{b}	83^{b}	87^{b}	86^{c}
P-Value		0.001	0.003	<0.0001	<0.0001
SEM		0.48	0.43	0.6	0.6
Interactions					
NL*Ca: P (2:1) *oil	1	81	88	91	94
	1.5	79	88	91	94
	2	78	86	80	85
	2.5	75	82	86	86
PL*Ca: P (2:1) *oil	1	84	89	93	96
	1.5	81	89	94	97
	2	80	88	93	96
	2.5	77	85	86	88
NL*Ca :P (1.8:1)*oil	1	81	86	94	93
	1.5	80	86	94	93
	2	78	85	91	84
	2.5	75	82	90	85
PL*Ca: P(1.8:1)*oil	1	79	89	95	96
	1.5	82	85	95	96
	2	82	87	95	96
	2.5	79	84	90	87
NL*Ca :P (1.6:1)*oil	1	84	85	86	92
	1.5	84	84	86	92
	2	82	86	78	83
	2.5	78	82	85	84
PL*Ca: P(1.6:1)*oil	1	83	89	92	95
	1.5	86	86	92	95
	2	85	87	91	95
	2.5	82	84	85	86
P-Value		0.9	0.9	0.2	1
SEM		0.48	0.43	0.6	0.6

NL: negative Lipidol; PL: positive Lipidol; Ca:P: calcium into phosphorus ratio; SEM: standard error mean. a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

Table 8. The effect of emulsifier supplementation with different calcium levels on serum lipid profile in broilers.

		Starter					Grower					Finisher				
		Chol	TAG	HDL	LDL	VLDL	Chol	TAG	HDL	LDL	VLDL	Chol	TAG	HDL	LDL	VLDL
Addition of Emulsifier																
With Lipidol (PL)		131	55	48	68	11	114	70	50	50	14	140	33	51	83	7
Without Lipidol (NL)		126	54	50	70	11	119	73	48	56	15	126	38	49	70	8
P-Value		<0.0001	0.06	0.007	0.048	0.7	0.003	0.2	0.06	<0.0001	0.4	<0.0001	<0.0001	0.2	<0.0001	0.2
SEM		6.4	4.7	1.3	6.5	1.2	7.6	6.7	1.3	7.5	1.7	10.7	3	1.6	10.8	1.1
Calcium levels (Ca: P)																
2:01		128	55	51	65	11	124	80	50	58	16	145	37	51	88	8
1.8:1		123	46	50	63	9	112	69	49	48	14	142	31	50	86	6
1.6:1		135	62	45	79	12	114	66	48	52	13	111	39	48	56	8
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	0.007	<0.0001	<0.0001	0.4	<0.0001	0.2	<0.0001	<0.0001	0.1	<0.0001	0.5
SEM		2.6	1.9	0.5	2.6	0.5	3.1	2.7	0.5	3	0.7	4.4	1.2	0.7	4.4	0.5
Addition of oil%																
1		134^{a}	49^{b}	49	75^{a}	10	132^{a}	85^{a}	49	66^{a}	17	141^{a}	37	52	83^{a}	7
1.5		120^{b}	53^{ab}	50	68^{b}	11	95^{b}	68^{b}	50	30^{c}	14	120^{c}	37	50	64^{c}	7
2		136^{a}	58^{a}	49	69^{b}	12	133^{a}	71^{b}	49	70^{a}	14	130^{b}	33	50	74^{b}	7
2.5		124^{b}	57^{a}	48	64^{bc}	11	106^{b}	64^{b}	48	45^{b}	13	141^{a}	37	49	85^{a}	7
P-Value		<0.0001	<0.0001	0.05	<0.0001	0.4	<0.0001	<0.0001	0.6	<0.0001	0.07	<0.0001	0.1	0.4	<0.0001	0.9
SEM		2.6	1.9	0.5	2.6	0.5	3.1	2.7	0.5	3	0.7	4.4	1.2	0.7	4.4	0.5
Interactions																
NL*Ca : P (2:1)*oil	1	146^{cd}	55^{cd}	49^{cde}	86^{ef}	11^{bcd}	129^{cd}	95^{abc}	47	63^{c}	19^{abc}	133^{f}	44^{bc}	56	78^{e}	9
	1.5	134^{ef}	38^{gh}	51^{abcd}	75^{g}	8^{bcd}	98^{e}	74^{defgh}	50	33^{fg}	15^{abcde}	104^{ij}	35^{cdef}	50	47^{g}	7
	2	129^{f}	66^{b}	53^{ab}	63^{ij}	13^{abc}	155^{a}	69^{fgh}	49	92^{a}	14^{abcde}	100^{j}	49^{b}	51	39^{g}	10
	2.5	106^{ij}	50^{cde}	52^{abc}	44^{m}	10^{bcd}	122^{cd}	86^{bcdef}	50	55^{cd}	17^{abcde}	242^{a}	21^{h}	51	187^{a}	4
PL*Ca : P (2:1)*oil	1	139de	54^{cd}	54^{a}	74^{gh}	11^{bcd}	157^{a}	99^{ab}	48	89^{a}	20^{ab}	188^{b}	43^{bcd}	53	126^{b}	9
	1.5	152^{bc}	68^{b}	47^{efg}	91de	14^{ab}	122^{cd}	79^{cdefg}	51	55^{cd}	16^{abcde}	151^{d}	40^{bcde}	45	98^{c}	8
	2	111^{hi}	52^{cd}	53^{ab}	48^{lm}	10^{bcd}	129^{cd}	99^{ab}	51	58^{cd}	20^{ab}	147de	36^{cdef}	51	89^{cd}	7
	2.5	103^{j}	56^{c}	52^{abc}	37^{n}	11^{bcd}	78^{g}	40^{k}	54	16^{i}	8^{e}	98^{jk}	32^{defg}	54	38^{g}	6
NL*Ca :P(1.8:1)*oil	1	108^{hij}	52^{cd}	52^{abc}	46^{m}	10^{bcd}	124^{cd}	80^{cdefg}	51	57^{cd}	16^{abcde}	117^{gh}	36^{cdef}	52	58^{f}	7
	1.5	95^{k}	34^{hi}	54^{a}	34^{n}	7^{cd}	99^{e}	45^{jk}	51	35^{ef}	13^{abcde}	89^{kl}	37^{cde}	53	28^{h}	8
	2	163^{a}	45^{ef}	49^{cde}	105^{ab}	9^{bcd}	156^{a}	72^{efgh}	46	96^{a}	14^{abcde}	140^{ef}	22^{gh}	48	88^{cd}	4
	2.5	136^{e}	41^{fg}	48^{def}	80^{fg}	8^{bcd}	118^{d}	60^{hij}	49	57^{cd}	12^{bcde}	144de	30^{efgh}	48	90^{cd}	6

Table 8. Continue

PL*Ca : P(1.8:1)*oil	1	111^{hi}	32^{i}	51^{abcd}	54^{kl}	6^{d}	99^{e}	111^{a}	53	24^{ghi}	22^{a}	183^{b}	35^{cdef}	52	124^{b}	7
	1.5	118^{g}	66^{b}	49^{cde}	56^{k}	13^{abc}	130^{c}	39^{k}	50	72^{b}	8^{e}	151^{d}	22^{gh}	54	93^{c}	4
	2	110^{hi}	31^{i}	48^{def}	56^{k}	6^{d}	92^{ef}	74^{defgh}	51	26^{fgh}	15^{abcde}	146de	25^{fgh}	52	89^{cd}	5
	2.5	141de	66^{b}	50^{bcde}	78^{g}	13^{abc}	76^{g}	70^{fgh}	43	19^{hi}	14^{abcde}	169^{c}	44^{bc}	43	117^{b}	9
NL*Ca :P(1.6:1)*oil	1	115^{gh}	49de	51^{abcd}	54^{kl}	10^{bcd}	123^{cd}	88^{bcde}	53	52^{d}	18^{abcd}	140^{ef}	29^{efgh}	53	81de	6
	1.5	77^{l}	91^{a}	53^{ab}	58^{jk}	18^{a}	66^{h}	52^{ijk}	49	57^{cd}	10^{cde}	83^{l}	40^{bcde}	50	25^{h}	8
	2	150^{c}	52^{cd}	43^{h}	97^{cd}	10^{bcd}	129^{cd}	103^{ab}	48	60^{cd}	21^{ab}	120^{gh}	44^{bc}	43	68^{f}	9
	2.5	146^{cd}	45^{ef}	38^{i}	99^{bc}	9^{bcd}	147^{ab}	45^{jk}	41	97^{a}	9de	72^{m}	62^{a}	40	20^{h}	12
PL*Ca : P(1.6:1)*oil	1	157^{ab}	45^{ef}	39^{i}	109^{a}	9^{bcd}	141^{b}	90^{bcd}	48	75^{b}	18^{abcd}	122^{g}	40^{bcde}	48	66^{f}	8
	1.5	152^{bc}	55^{cd}	44^{gh}	97^{cd}	11^{bcd}	84^{fg}	45^{jk}	47	28^{fgh}	9de	111^{hi}	33^{cdef}	49	60^{f}	7
	2	143^{d}	66^{b}	45^{fgh}	49^{lm}	13^{abc}	99^{e}	42^{k}	49	42^{e}	8^{e}	123^{g}	25^{fgh}	50	68^{f}	5
	2.5	136^{e}	96^{a}	49^{cde}	68^{hi}	19^{a}	121^{cd}	66^{ghi}	51	57^{cd}	13^{abcde}	117^{gh}	36^{cdef}	52	58^{f}	7
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7	<0.0001	0.007	<0.0001	<0.0001	0.9	<0.0001	0.8
SEM		2.6	1.9	0.5	2.6	0.5	3.1	2.7	0.5	3	0.7	4.4	1.2	0.7	4.4	0.5

NL: negative Lipidol; PL: positive Lipidol; Ca:P: calcium into phosphorus ratio; Chol: cholesterol; SEM: standard error mean. a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

In terms of HDL, there was no significant difference among all treatments. Concerning LDL, the group given the 1.6:1 Ca to P treatment without an emulsifier with 2.5% oil showed the highest LDL, whereas utilization of the 2:1 Ca to P treatment with an emulsifier with 2.5% oil showed the lowest LDL. Regarding VLDL, the group given the 1.8:1 Ca to P treatment with emulsifier supplementation with 1% oil showed the highest level of VLDL and significantly more than that treated with the same Ca level without an emulsifier with 2.5% oil. Moreover, there was no significant difference among other groups in VLDL during the grower period.

During the finisher period, concerning cholesterol, the group given the 2:1 Ca to P treatment without emulsifier supplementation with 2.5% oil showed the highest cholesterol level, followed by that treated with the same Ca level with an emulsifier with 1% oil. However, the group given the 1.6:1 Ca to P treatment without emulsifier supplementation with 2.5% oil showed the lowest cholesterol level. Concerning TAG, the group given the 1.6:1 Ca to P treatment without emulsifier supplementation with 2.5% oil showed the highest level of TAG, followed by the group given the 2:1 Ca to P treatment without emulsifier supplementation with 2% oil, which was more significant than the group treated with 1.5% oil level. The latter group has more TAG than the group given the 1.8:1 Ca to P treatment without emulsifier supplementation with 2% oil. There was no significant difference in TAG among others during the finisher stage.

Concerning LDL, the group given the 2:1 Ca to P treatment without emulsifier supplementation with 2.5% oil showed the highest LDL level, followed by the group given the same Ca level with 1% oil with emulsifier supplementation, and then the group was given 1.5% oil. The latter had significantly more LDL than the group given the 1.6:1 Ca to P at treatment without emulsifier supplementation with 1% oil, which is more than that in the group given the same treatment with 2% oil. The latter treatment significantly increased LDL compared to the 2:1 Ca to P treatment without emulsifier supplementation with 1.5% oil, and there was no significant difference among the other groups in LDL during the finisher period. Concerning HDL and VLDL, there was no significant difference among all treatments during the finisher stage.

Minerals Deposition

Results (Table 9) showed that during the starter, grower, and finisher stages, emulsifier supplementation significantly increased Ca and P deposition in the chick body but significantly reduced Mn deposition. In the grower stage, there was no significant difference in Mn by an emulsifier. During the starter and grower periods, the addition of Ca and P at 2:1 and 1.8:1 significantly increased the Ca and P deposition more than the 1.6:1 Ca and P rate and showed the lowest Mn level in the body. Low oil levels (1% and 1.5%) showed the highest Ca level and 1% oil level showed the highest P level and the lowest level of Mn during all stages.

By interaction, during the starter period, groups given a 2:1 Ca:P diet with an emulsifier with 1.5% oil showed the highest Ca levels, followed by the group given 2:1 Ca:P with 1% oil without emulsifier supplementation, which significantly increased Ca deposition in the body more than the 1.8:1 Ca:P treatment 2% oil with emulsifier supplementation. Moreover, the 1.6:1 Ca:P treatment with 2% oil with emulsifier supplementation resulted in the lowest Ca level. Regarding phosphorus, groups fed a 2:1 Ca:P diet with an emulsifier with 1% oil and a 1.8:1 Ca:P diet with 2.5% oil with an emulsifier showed the highest P levels deposited in the body, whereas the group given a 1.6:1 Ca:P diet with 2.5% oil

Table 9. The effect of emulsifier supplementation with different calcium levels on minerals in broilers.

		Starter (Whole body chicks)			Grower (Whole body chicks)			Finisher (Tibia)		
		Ca%	P%	Mn%	Ca%	P%	Mn%	Ca%	P%	Mn%
Addition of Emulsifier										
With Lipidol (PL)		7.6	5.97	0.03	11.37	7.87	0.05	22.22	14.7	0.12
Without Lipidol (NL)		7.2	5.68	0.04	10.38	7.65	0.054	21.88	14.3	0.18
P-Value		<0.0001	<0.0001	0.04	<0.0001	<0.0001	0.09	<0.0001	<0.0001	<0.0001
SEM		0.55	0.31	0.005	0.71	0.38	0.007	0.4	0.3	0.01
Calcium levels (Ca: P)										
2:01		7.86^{a}	6.31^{a}	0.03^{b}	11.76^{a}	8.31^{a}	0.05^{b}	22.57^{a}	15.06^{a}	0.12^{c}
1.8:1		7.8^{a}	5.82^{a}	0.02^{b}	11.13^{a}	7.61^{b}	0.035^{c}	22.8^{a}	14.3^{b}	0.15^{b}
1.6:1		6.5^{b}	5.34^{b}	0.05^{a}	9.73^{b}	7.37^{b}	0.07^{a}	20.8^{b}	14.12^{b}	0.17^{a}
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.05	<0.0001
SEM		0.27	0.15	0.003	0.36	0.18	0.003	0.2	0.15	0.008
Addition of oil%										
1		7.9^{a}	6.77^{a}	0.028^{b}	11.79^{a}	9^{a}	0.04^{b}	22.3^{a}	15.35^{a}	0.13^{b}
1.5		8.05^{a}	5.57^{b}	0.03^{b}	11.66^{a}	7.75^{b}	0.05^{ab}	22.5^{a}	14.53^{b}	0.14^{ab}
2		6.77^{b}	5.84^{b}	0.04^{a}	10.27^{b}	7.57^{bc}	0.05^{ab}	22^{ab}	14.33^{b}	0.17^{a}
2.5		6.83^{b}	5.12^{b}	0.04^{a}	9.77 ^{b}	6.74^{c}	0.06^{a}	21.43^{b}	13.81^{b}	0.14^{ab}
P-Value		<0.0001	<0.0001	0.003	<0.0001	<0.0001	0.002	<0.0001	<0.0001	<0.0001
SEM		0.27	0.15	0.003	0.36	0.18	0.003	0.2	0.15	0.008
Interactions										
NL*Ca: P (2:1)*oil	1	10.44^{b}	6.31^{ef}	0.04^{de}	9.66^{l}	8.31^{ef}	0.06^{de}	25.4^{b}	16.63^{a}	0.18^{d}
	1.5	5.92^{k}	5.98^{fgh}	0.01^{g}	14.17^{c}	9.87^{c}	0.02^{hi}	20.96^{i}	14.02^{de}	0.16^{e}
	2	8.56^{ef}	7.87^{ab}	0.02^{fg}	12.31^{fg}	7.98^{fg}	0.03^{gh}	22.1^{ef}	12.95^{g}	0.16^{e}
	2.5	6.22^{jk}	6.15^{efg}	0.05^{cd}	9.96^{l}	8.15^{efg}	0.07^{cd}	21.83^{fg}	14.08^{de}	0.08^{i}
PL*Ca: P (2:1)*oil	1	8.92^{e}	8.31^{a}	0.03^{ef}	16.18^{a}	10.31^{b}	0.05^{ef}	21.63^{gh}	16.65^{a}	0.08^{i}
	1.5	11.1^{a}	5.94^{fgh}	0.02^{fg}	12.65^{f}	7.22^{i}	0.04^{fg}	24.69^{c}	14.03^{de}	0.06^{j}
	2	6.8^{i}	5.22^{ij}	0.03^{ef}	10.53^{k}	7.94^{g}	0.04^{fg}	22.11^{ef}	16.64^{a}	0.16^{e}
	2.5	4.96l	4.7^{kl}	0.06^{bc}	8.62^{m}	6.7^{j}	0.07^{cd}	21.83^{fg}	15.55^{b}	0.06^{j}
NL*Ca :P (1.8:1)*oil	1	8.13^{fg}	6.45^{de}	0.01^{g}	13.28^{e}	8.45^{e}	0.01^{i}	22.37^{e}	14de	0.1^{h}
	1.5	9.54^{d}	5.2^{ij}	0.05^{cd}	11.86^{hi}	7.59^{h}	0.07^{cd}	21.71^{gh}	15.12^{c}	0.21^{b}
	2	6.62^{ij}	5.59^{hi}	0.02^{fg}	10.35^{k}	7.2^{i}	0.02^{hi}	21.69^{gh}	12.93^{g}	0.16^{e}
	2.5	4.36^{mn}	3.88^{no}	0.04^{de}	3.74^{p}	4.72^{n}	0.06^{de}	22.43^{e}	14.21^{d}	0.2^{bc}
PL*Ca: P(1.8:1)*oil	1	4.78^{lm}	6.23^{efg}	0.02^{fg}	12.2^{gh}	8.22^{efg}	0.01^{i}	22.39^{e}	16.43^{a}	0.11^{gh}
	1.5	8.46^{ef}	6.77^{cd}	0.02^{fg}	8.66^{m}	5.87^{l}	0.04^{fg}	24.16^{d}	14.07^{de}	0.13^{f}
	2	9.99^{c}	4.42^{lm}	0.02^{fg}	13.78^{d}	8.78^{d}	0.04^{fg}	21.71^{gh}	15.41^{bc}	0.16^{e}
	2.5	10.48^{b}	8.07^{a}	0.02^{fg}	15.22^{b}	10.06^{bc}	0.03^{gh}	25.68^{b}	12.48^{h}	0.16^{e}
NL*Ca :P (1.6:1)*oil	1	7.31^{h}	7.5^{b}	0.02^{fg}	6.81^{o}	10.79^{a}	0.02^{hi}	20.55^{j}	14.2^{d}	0.12^{fg}
	1.5	4.4^{mn}	4.31^{lmn}	0.06^{bc}	11.04^{j}	7.1^{i}	0.08^{bc}	21.69^{gh}	16.27^{a}	0.2^{bc}
	2	4.37^{mn}	5.1^{jk}	0.08^{a}	7.06^{o}	6.31k	0.1^{a}	94.78^{a}	13.93^{de}	0.29^{a}
	2.5	10.58^{b}	3.79^{o}	0.08^{a}	14.32^{c}	5.32^{m}	0.1^{a}	18.19^{l}	13.27^{fg}	0.3^{a}
PL*Ca: P(1.6:1)*oil	1	7.88^{g}	5.85^{gh}	0.05^{cd}	12.66^{f}	7.86^{gh}	0.07^{cd}	21.36^{h}	14.2^{d}	0.19^{cd}
	1.5	8.93^{e}	5.22^{ij}	0.04^{de}	11.61^{i}	8.87^{d}	0.06^{de}	21.63^{gh}	13.67^{ef}	0.11^{gh}
	2	4.2^{n}	6.87^{c}	0.07^{ab}	7.61^{n}	7.22^{i}	0.09^{ab}	20.75^{ij}	14.17^{d}	0.13^{f}
	2.5	4.38^{mn}	4.13^{mno}	0.01^{g}	6.75^{o}	5.47^{m}	0.02^{hi}	18.68^{k}	13.28^{fg}	0.04^{k}
P-Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SEM		0.27	0.15	0.003	0.36	0.18	0.003	0.2	0.15	0.008

NL: negative Lipidol; PL: positive Lipidol; Ca:P: calcium into phosphorus ratio; Mn: manganese; SEM: standard error mean. a–d values within the column of each classification with different letters are significantly different ($P < 0.05$).

without emulsifier showed the lowest level of P. In terms of Mn, groups treated with a 1.6:1 Ca:P diet and 2% and 2.5% oil levels without an emulsifier showed the highest level of Mn deposition. Furthermore, groups treated with a 2:1 Ca:P with 2.5% oil with emulsifier supplementation had significantly more elevated Mn deposition than those given 1% oil without an emulsifier with the same Ca level. Nevertheless, there was no significant difference among the others in Mn levels.

By interaction, during the grower stage, the group given a 2:1 Ca:P treatment with emulsifier supplementation with 1% oil showed the highest Ca deposition level in the body, followed by that given a 1.8:1 Ca:P treatment with 2.5% oil with an emulsifier. The group given a 1.8:1 Ca:P treatment without emulsifier supplementation with 2.5% oil showed the lowest Ca level. In terms of P, groups treated with 1.6:1 Ca:P with 1% oil without emulsifier supplementation showed the highest P level deposited in the body, which significantly increased P compared to the P level in the group given the 2:1 Ca:P treatment with 1% oil with emulsifier supplementation followed by that given the same treatment with 1.5% oil without emulsifier supplementation. Groups treated with a 1.8:1 Ca:P diet with 2.5 % oil without emulsifier showed the lowest P level. Concerning Mn deposition, the highest level of Mn deposited was observed in birds treated with 1.6:1 Ca:P with the highest oil level of 2% or 2.5% without emulsifier supplementation, which was significantly more than that in the 1.5% oil group. The latter significantly increased Mn deposition compared to the 1.8:1 Ca:P treatment with 2.5% oil without emulsifier supplementation, 1.6:1 with 1.5% oil with an emulsifier, and 2:1 with 1% oil without emulsifier. The latter treatment significantly increased Mn deposition compared to 1.5% or 2% oil treatment with an emulsifier. The lowest level of Mn deposition was observed in the group given a diet with 1.8:1 Ca:P with 1% oil with or without emulsifier. Furthermore, there was no significant difference among the other groups.

By interaction, during the finisher stage, the 1.6:1 Ca:P treatment with 2 % oil without emulsifier supplementation resulted in the highest level of Ca, which was significantly more than the 1.8:1 Ca:P with 2.5% oil with an emulsifier. Furthermore, the group given 1.6:1 Ca:P with 2.5% oil without an emulsifier showed the lowest Ca level. Concerning the P deposition, emulsifier supplementation in groups treated with 2:1 Ca:P with 2% oil significantly resulted in the highest level of P deposition in the tibia, followed by that treated with the same treatment with 2.5% oil. The latter treatment significantly increased P compared to the 1.8:1 Ca: P treatment with 1.5% oil without an emulsifier, followed by the same treatment with 2.5% oil. P deposition significantly increased with a 1.6:1 Ca: P treatment with an emulsifier with 1.5% oil, which was more than the P level with 2:1 Ca:P with 2% oil without an emulsifier. The 1.8:1 Ca:P treatment with 2.5% oil with an emulsifier resulted in the lowest level of P in the tibia. Concerning Mn deposition, the 1.6:1 Ca: P treatment with the highest levels of oil (2% or 2.5%) without emulsifier supplementation showed the highest level of Mn deposited, which was more than the levels in the groups treated with 1.8:1 Ca:P with 1.5% oil without emulsifier. The latter treatment significantly increased Mn compared to the level in the group given 1.6:1 Ca:P with 1% oil with emulsifier supplementation. The 1.6:1 Ca:P treatment with oil 2.5% with emulsifier supplementation resulted in the lowest level of Mn in the tibia.

DISCUSSION

In the absence of studies demonstrating the interaction between emulsifiers and calcium levels on broiler performance, we had to compare our findings with the results related to emulsifiers and calcium separately. Improved BW and BWG in our study due to emulsifier supplementation supported the hypothesis that emulsifiers would improve the performance of broiler chickens by enhancing nutrient digestibility, especially fat digestibility. In our results, low calcium levels increased the performance during all periods, which agreed with the results of Sebastian *et al*. (1996), who reported that the higher dietary Ca level significantly reduced the performance. Moreover, Hamdi *et al*. (2015) have found that a low level of Ca 0.7% increased BWG compared to a high Ca level of 0.9%; however, Rama Rao *et al*. (2006) reported no differences in BWG on day 14 by different Ca levels. Our results agree with those of Allahyari-Bake and Jahanian (2017) who have found that supplementation of lysophosphatidylcholine in a broiler diet elevated average daily weight gain when soy-free fatty acids were used, indicating that the energy of diets containing a high level of free fatty acid (FFA) was not utilized as effectively as the energy in diets with a low FFA level. Tabeidian *et al*. (2010) have concluded that carcass weight increased to a greater extent by elevating dietary fat but was not affected by a higher dietary Ca intake alone. There was no significant effect on carcass weight and feed intake ($P > 0.05$) by high dietary Ca inclusions; however, a combination of 1% or 2% of Ca and 2% of animal fat improved broiler performance and is considered a good management practice in Ross 308 strain diets.

In our results, a low Ca level resulted in the lowest feed intake, unlike the results of Hamdi *et al*. (2015), who reported that a low Ca level of 0.7% led to higher feed intake than a high Ca level of 0.9% during the starter period and that feed intake was not affected by oil levels. Our findings are not in the line with those of Smith *et al*. (2003), who studied the effect of a diet with fat and different calcium levels on poultry performance and showed that the addition of 1.5% calcium with fat reduced feed intake compared to 0.93% calcium. Velasco *et al*. (2010) have found that chicks fed an unsaturated fat diet had better feed efficiency than those fed a saturated fat diet. At an early phase, unsaturated fat resulted in better intestinal absorption compared to saturated ones. Higher unsaturated fatty acids (UFAs) containing fat increase bile secretion and lipase activity, in turn, improving the digestibility of young chickens (Noy and Sklan, 1996). This finding agrees with those of Mossab *et al*. (2000), who reported lower bile secretion and lipase activity due to the low-fat utilization in the first week of broilers' age. In our results, the positive effects on growth performance were also due to an increase in nutrition utilization and metabolizable energy in broilers through enhancing the digestion of soybean oil by emulsifier supplementation.

Crespo and Esteve-García (2002) have studied the effect of four types of fat animal fat, olive oil, sunflower oil, and flax oil with two lipid levels (6% and 10%) in the diet of the male chicks and found that dietary fat significantly reduced feed intake. Moreover, feed efficiency was affected by different levels of fat. Similarly, Maertens *et al*. (2015) have found that FCR was improved in the group supplemented with emulsifiers compared to the control. Our results agree with those of Hulan *et al*. (1984) and Scheideler and Baughman (1989), who have reported that dietary fats improved FCR due to the secretion of cholecystokinin hormone and delayed transit time of food in the gastrointestinal tract, thus prolonging the presence of enzymes and subsequently improving its digestion. Our result showed that the group given the 1.6:1 Ca to P treatment with emulsifier with 2% oil had the lowest FCR, which agrees with the results of Kulkarni *et al*. (2019), who have demonstrated that the addition of emulsifiers improved broiler's feed efficiency and performance that may lead to lower feed intake and costs.

Dressing and abdominal fat percentage were also improved by emulsifier supplementation. Abdominal fat percentage was significantly elevated by a low Ca level (1.6:1). Similarly, the findings of Talpur *et al*. (2012) found that emulsifier supplementation significantly increased abdominal fat percentage and dressing percentage was improved in broilers given a diet containing low Ca level (10 g/kg DM) compared with those fed higher Ca levels

(20 and 30 g/kg DM). The emulsifier may enhance the utilization of soybean oil for muscle formation and deposition of abdominal fat, as confirmed by the findings of a previous study (Roy *et al.*, 2010). Current results are in line with those of Hakansson (1975) and Sibbald and Price (1977), who have shown that consuming a diet high in fat and calcium decreases abdominal fat. Dale and Fuller (1979) have reported that a high dietary fat in the broiler diet can increase the carcass fat. Sanz *et al.* (2000) have reported that a diet rich in saturated fats increased abdominal fat in broilers compared with diets containing polyunsaturated fatty acids. Raju *et al.* (2011) have demonstrated that, on day 35, adding rice bran lysolecithin at rates of 2.5% and 5% to the diet reduced abdominal fat percentage in broilers. Moreover, Valable *et al.* (2017) reported that birds given low dietary Ca (0.60%) had a higher body fat content than those given higher dietary Ca (0.90%) on day 21. Our results are not in line with the previous findings of Tabeidian *et al.* (2010), who have found that increasing the level of Ca and fat elevated the amount of abdominal fat by 2% dietary fat resulting in a higher abdominal fat percentage. Furthermore, carcass weight was reported to increase to a greater extent by elevating dietary fat but was not affected by a higher dietary Ca intake alone. Siyal *et al.* (2017) have found that soy lecithin at a rate of 0.10 recorded the highest relative liver weight compared to soy lecithin at a rate of 0.05 and the control group without soy lecithin ($P < 0.05$). Moreover, bursa, spleen, pancreas, and thymus relative weights were not affected by the different dietary treatments during days 21 and 42. Nagargoje *et al.* (2016) found that the addition of soy lecithin into broilers' diet resulted in better liver weight. The liver is the main organ involved in the lipid metabolism of the body, accounting for 95% of the de novo fatty acid synthesis in birds (Theil and Lauridsen, 2007).

The results of improved fat digestibility during all periods and improved DM digestibility during the finisher phase are in line with those of Mathlouthi *et al.* (2002), who have found that the emulsifier improved nutrient digestibility; thus, the bird gets its energy needs, and therefore, feed intake is not increased. Moreover, the findings of Kaczmarek *et al.* (2015) demonstrated that emulsifier supplementation enhanced fat digestibility on day 14, but when feed intake was taken into consideration, dietary fat utilization was elevated only by 1.2 g per bird over the entire 14-day period. Calcium with free saturated fatty acids may form soap precipitates, thus reducing the fat digestibility (Pepper *et al.*, 1955; Edwards *et al.*, 1960) and also interacting with inorganic phosphorus in the gastrointestinal tract (Hurwitz and Bar, 1971), which may suggest that diets should be higher in Ca and P to avoid this problem.

A higher Ca level (1.5%) significantly decreased BWT and apparent digestibility of crude protein, fat, organic matter, and phosphorus compared to lower Ca levels (1% and 1.25%); however, higher Ca, P, and ash digestibility was reported in the birds given 1.25% Ca than that given 1 % Ca (Abdulla *et al.*, 2016). Emulsifier supplementation increased fat digestibility in the current study, which is in agreement with the studies of Zhao *et al.* (2015) and Upadhaya *et al.* (2017), who have reported that the addition of emulsifier improved nutrient digestibility in weaning pigs. Furthermore, Jansen (2015) has found that fat digestibility, DM digestibility, and the apparent metabolizable energy in young broilers fed the basal diet with lysolecithin were higher than in those fed the basal diet without lysolecithin.

Crespo and Esteve-García (2003) and Aguilar *et al.* (2011) agreed with our results when they found that the supplementation of a diet with high levels of saturated fatty acids increased the levels of TAG and disagreed with our results as they found that VLDL and LDL were increased by high levels of fatty acid, which also reduced the levels of HDL in broilers. During the finisher period, the emulsifier significantly increased total cholesterol and LDL and decreased TAG. These findings agree with those of Tidwell *et al.* (1957), who have reported that fat absorption was improved in certain abnormal conditions and chylomicronemia increased by administration of emulsifier (polyoxyethylene sorbitan monooleate), supporting the idea that the emulsifier might influence the absorption of fat-like substances. They have also reported that the presence of bile and fat in the chyme has long been known to improve cholesterol absorption. The exogenous emulsifier may act as a natural emulsifier and may play an important role in fat emulsification, digestion, and absorption; thus, the mechanism of increasing cholesterol by Lipidol may be attributed to this reason. Furthermore, the emulsifier helps dissolve FFAs, which are hardly soluble in bile salt micelle alone, thus improving the digestibility of saturated fatty acids and deposition of fatty acids in the body tissue (Roy *et al.*, 2010).

Neto *et al.* (2011) and Aguilar *et al.* (2013) have found that the exogenous emulsifier did not affect the levels of cholesterol, triglyceride, LDL, HDL, and VLDL in broiler chicken. Shearer *et al.* (2012) have found that total serum cholesterol, TAG, and VLDL were reduced by including polyunsaturated fatty acid- (PUFA-) rich oil in broiler diets compared to saturated fatty acid-rich oil.

Emulsifier supplementation significantly increased calcium and phosphorus deposition in the chick body. However, it significantly decreased manganese deposition during all stages, which is in agreement with the findings by Jones *et al.* (1992) and Dierick and Decuypere (2004), who have reported that calcium and phosphorus absorption was improved by emulsifier supplementation. However, contrasting results are also present, such as the findings by Øverland and Sundstøl (1995).

High Ca and P rates (2:1 and 1.8:1) significantly increased Ca and P deposition compared to low Ca and P rates (1.6:1). Ca interact with inorganic phosphorus in the gastrointestinal tract (GIT) to form insoluble Ca orthophosphate (Plumstead *et al.*, 2008), which may also decrease the solubility of inorganic phosphorus. Thus, the Ca level should be increased in the diet. That is unlike the findings of Browning *et al.* (2012), who found that decreasing dietary concentrations of Ca to available P was associated with elevated Ca retention efficiency compared to high Ca to available P diets, which explains the broilers' physiological response to overcome a Ca deficiency by upregulating its nutrient transfer and deposition infrastructure. The results of Li *et al.* (2017) indicated that the requirements of Ca and P for broilers are lower than those recommended by NRC (1994). Moreover, they have found that the bird performance was adversely affected by higher dietary Ca concentrations and lower dietary P concentrations without phytase supplementation as the phytase alleviated this negative effect. Higher dietary calcium levels may affect bone formation and reduce chick growth during the early period of growth (Hamdi *et al.*, 2015).

Low oil levels (1% and 1.5%) resulted in the highest level of Ca deposition and 1% oil level led to the highest P level and the lowest Mn level during all stages. These findings agree with Roy *et al.* (2010), who reported that the group given an emulsifier at a low dose of fat (1%) in the diet showed a higher apparent Cu absorption than that of the control one and that given an emulsifier at a high dose of fat (2%) in the diet. Moreover, Valable *et al.* (2017) have reported that low dietary Ca (0.6%) significantly resulted in higher Ca in the tibia than higher dietary Ca (0.9%) on day 21. Our result agrees with Shafy and McDonald (1990; 1991), who has investigated that elevating dietary Ca leads to a defect in the absorption of other minerals, especially Mn, Mg, and Zn; thus, Mn absorption and deposition in our results may be reduced by high Ca level in the diet. Venäläinen *et al.* (2006) have found that elevated Ca and available P levels in the broiler diet significantly improved the content of tibia ash; however, they did not affect the breaking strength of the tibia. Tabeidian *et al.* (2010) have demonstrated that bone ash percentage was not significant by adding different fat levels to broiler diets. Williams *et al.* (2000) have reported that lower bone ash content was observed in fast-growing strains than in slow-growing ones, which may suggest that Ca and P should be higher in the diet than the current recommendations of 10 g/kg for Ca and 4.5 g/kg for nonphytate P at first 21 days of age (NRC, 1994) to improve skeletal integrity of fast-growing strains (Hamdi *et al.*, 2015). Our results are not similar to the findings of Roy *et al.* (2010), who reported that the minerals intake, excretion, and absorption were mostly unaffected by emulsifier supplementation in the diet. Chen *et al*. (2019) have explained the positive effects mechanism of emulsifiers on the bones of broilers and described the ability of emulsifiers in promoting intestinal development by increasing absorption surface area and claudin-3 expression; thus, the emulsifier increased nutrient availability in the diets, which led to beneficial effects on bone quality.

CONCLUSION

Lipidol supplementation with low calcium and high oil levels (2% and 2.5%) in diets positively affected growth performance. Furthermore, dietary supplementation of Lipidol with low calcium (1.6:1) and oil levels improved digestibility during the starter period only; moreover, the emulsifier showed positive effects on bone quality with high calcium levels in a diet. Lipidol could become an effective feed additive for broilers to achieve better production.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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