Effects of an emulsifier blend supplementation on growth performance, nutrient digestibility, intestinal morphology, and muscle fatty acid profile of broiler chickens fed with different levels of energy and protein

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ABSTRACT The effects of emulsifier blend (**EB**) supplementation of diets with various levels of metabolizable energy (**ME**) and crude protein (**CP**) on broiler performance, digestibility, gut morphology, and muscle fatty acid profile were investigated over a 42-d period. Diets were arranged factorially (2 × 2 × 3) and consisted of 2 levels of ME (normal [commercially recommended levels] and low [100 kcal/kg reduction in dietary ME]), 2 levels of CP and limiting amino acids (normal [commercially recommended levels] and low [95% of the normal CP level]), and 3 levels of EB supplementation (0, 1, and 2 g/kg of diet). A total of 1,200 one-day-old male broiler chickens (Ross 308) were randomly assigned to 12 treatment groups (5 pens/treatment with 20 birds/pen). Supplemental EB linearly improved ($P < 0.05$) final body weight, overall average daily gain, and feed conversion ratio, but the magnitude of the responses was greater in low-ME and low-CP treatments, resulting in significant ME × CP × EB interactions. Similarly, the inclusion of EB in the diet, particularly at 2 g/kg, increased the ileal digestibility of crude protein and crude fat, as well as the AMEn value ($P < 0.05$), but the response was greater at lower ME concentration, indicating significant ME × EB interactions. Additionally, there were CP × EB interactions ($P < 0.05$) for duodenal villus height and villus height/crypt depth ratio, indicating that the effect of EB on these responses was more marked at lower dietary CP levels. An increase in dietary EB levels was accompanied by a linear increase in the concentration of total saturated fatty acids and a linear decrease ($P < 0.05$) in the concentrations of total polyunsaturated fatty acids in both breast and thigh meat. In conclusion, the positive effects of EB supplementation, particularly at a dietary inclusion level of 2 g/kg, were clearly evident in broiler chickens fed with low nutrient diets (−100 Kcal/kg ME and/or −5% CP and limiting amino acids) in terms of growth performance, nutrient digestibility, and gut morphology.

Key words: broiler performance, lysophospholipids, low nutrient diet, gut morphology, fatty acid composition

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INTRODUCTION

Metabolizable energy (**ME**) and crude protein (**CP**) are two of the most interesting and challenging topics for nutritionists because of their importance for poultry health and production (Kamran et al., 2008; Paraskeuas et al., 2016; Gous et al., 2018). Minimizing feed costs in poultry production requires new research on strategies to reduce ME and CP intake without compromising performance or health (Brickett et al., 2007). Increased fat digestibility may allow for lower inclusion levels of supplemental lipid and, as a result, a reduction in ME in the broiler chicken diet while maintaining the same level of performance, resulting in a reduction in feed production costs (Khonyoung et al., 2015; Majdolhosseini et al., 2019). This may be accomplished by using exogenous emulsifiers in poultry diets, which may help to overcome the physiological restrictions of the digestive tract in terms of digestibility of lipids and, to a lesser degree, other nutrients in poultry, especially in young birds (Zhao and Kim, 2017; Viñado et al., 2019). Previous studies on various poultry species found that supplementing diets with emulsifier agents, such as lysophospholipids and lysolecithins, compensated for the performance loss when dietary ME was reduced by 100 kcal/kg relative to optimum levels (Majdolhosseini et al., 2019; Haetinger et al., 2021; Nemati et al., 2021).

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Furthermore, lysophospholipid supplementation has been shown to improve the intestinal morphology of broiler chickens (Chen et al., 2019; Viñado et al., 2020). Although there have been some investigations into the antioxidant effects of lysophospholipids (Zangeneh et al., 2018; Taghavizadeh et al., 2020), there has been little evidence in the literature to date concerning the influence of lysophospholipids on the fatty acid composition of broiler muscle.

The natural surfactant lysophospholipids are derived from hydrolyzed soy lecithin and are created by the enzyme phospholipase A2, which breaks down phospholipids to release one hydrophobic fatty acid from each phospholipid chain (Joshi et al., 2006). Because this mechanism is vital and occurs naturally during fat digestion in birds, this technique can be employed to improve the performance of broiler chickens by adding exogenous lysophospholipids to their diet (Wealleans et al., 2020). When phospholipids are converted to lysophospholipids, their chemical properties are altered, which leads to enhanced emulsifying properties compared to lecithin and consequently more efficient fat hydrolysis (Boontiam et al., 2017). Higher hydrophilic-lipophilic balance values of lysophospholipids (2−12) compared to bile and lecithin result in a significant increase in the formation of smaller micelles in the guts and larger surface areas of lipid droplets, allowing pancreatic lipases to interact more efficiently (Hasenhuettl, 2008; Jansen et al., 2015). Lysophospholipids also affect the development of protein channels in the membrane by boosting ion exchanges (Maingret et al., 2000). Changes in deformation energy result in an increase in the number and size of membrane pores, which accelerates the transport of macromolecules across the cell membrane (Kelkar and Chattopadhyay, 2007). Both mechanisms aid in the transmission of nutrients, ranging from minute particles such as calcium ions to large components like polysaccharides that must be broken down in order to be absorbed, resulting in higher nutritional bioavailability and better broiler performance (Boontiam et al., 2017). According to Farjami et al. (2021), the digestion of proteins in an oil-in-water emulsion can be positively affected by physiological surfactants such as phosphatidylcholine and bile salts. In a previous study (Saleh et al., 2020), dietary supplementation with an emulsifier blend (phosphatidyl choline, lysophosphatidyl choline, and polyethylene glycol ricinoleate) increased both lipid and protein utilization. Haetinger et al. (2021) also observed that the product comprised of a synthetic emulsifier, monoglycerides, and lysophospholipids enhanced protein digestibility. Although lysophospholipids have been shown to improve nutrient utilization and digestion in broilers fed diets that are simultaneously low in ME and CP (Boontiam et al., 2017, 2019), there has been limited research examining the efficacy of emulsifiers when only low levels of dietary CP and amino acid are offered.

On the basis of previously reported background, it is hypothesized that lysophospholipids may improve the nutritional digestibility and metabolic condition of broilers fed diets with low levels of ME, CP, and limiting amino acids (**LAA**; i.e., methionine + cysteine, lysine, and threonine) in the feed, allowing their growth performance to be comparable to that of birds fed standard diets. Therefore, the first objective of this study was to determine whether an emulsifier blend (**EB**; containing 4 different lysophospholipids) can be used in ME- and/or CP-reduced diets to enable broilers to achieve comparable growth performance to birds fed standard control diets, thereby providing an opportunity to formulate low-cost diets. It was also determined whether there were any interactions between dietary ME levels, CP levels, and the use of emulsifier supplementation in the study. Growth performance, nutrient digestibility, gut morphology, and muscle fatty acid composition were used as the response criteria.

MATERIALS AND METHODS

The Animal Care and Use Committee of Ilam University (Ilam, Iran) approved all of the animal husbandry and experimental protocols used in the study (Contract number 98-271).

Experimental Design and Diets

A total of 1,200 male Ross 308 broiler chickens were purchased from a commercial hatchery for use in this project. In order to reduce variance in mean body weight (**BW**) within pens, broiler chickens were individually weighed upon arrival and then divided into 60 pens (12 treatments of 5 replicate groups of 20 birds). Throughout the growth stage, broiler chickens had unlimited access to water and feed. The lighting scheme was 24 h a day for the first 3 d, then lowered to 23 h of light afterwards. The initial room temperature was 34°C, and it was gradually reduced by 3°C each week until it reached 22°C at the start of wk 5. The relative humidity was maintained at 50 to 60% throughout the experiment.

This research used a 3-phase feeding program that included starter (0−10 d), grower (10−24 d), and finisher (24−42 d) diets. The experiment consisted of a 2 × 2 × 3 factorial arrangement of treatments, including 2 concentrations of ME (normal [3,000, 3,100, and 3,200 kcal/kg during starter, grower, and finisher periods, respectively], or low [2,900, 3,000, and 3,100 kcal/kg during starter, grower, and finisher periods, respectively]), 2 dietary CP and LAA levels (normal [commercially recommended levels] and low [95% of the normal CP level]), and 3 levels of EB supplementation (0, 1, and 2 g/kg of diet). Dietary CP levels in normal-CP diets were 230, 215, and 195 g/kg during the starter, grower, and finisher periods, respectively. The respective values were 218.5, 204.0, and 185 g/kg for low-CP diets. The feed components and chemical composition of the different experimental diets are shown in Tables 1 and 2. The emulsifier supplementation employed in this research (Artefier, Artevet.co., Wilmington, DE) was a natural multicomponent emulsifier that comprises 4 forms of lysophospholipids (lysophosphatidyl choline,

Table 1. Ingredients of the experimental diets with different levels of metabolizable energy (ME) and crude protein (CP) at any stage of growth.

	0−10 d				10−24 d				24−42 d			
ME level	Normal	Normal	Low	Low	Normal	Normal	Low	Low	Normal	Normal	Low	Low
CP level	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low
Ingredients (g/kg)												
Corn	581.5	604.7	546.7	569.8	614.8	636.9	581.3	603.5	658.6	678.6	626.3	646.7
Soybean meal	278.9	272.9	312.1	306.1	241.5	235.8	281.2	275.4	200.9	195.7	247.1	241.8
Corn gluten meal	77.6	61.0	53.1	36.5	77.1	61.3	49.1	33.3	68.3	53.8	36.8	22.2
Wheat bran	-	-	28.0	28.0	-	-	24.0	24.0	-	-	20.0	20.0
Soybean oil	12.0	12.0	12.0	12.0	21.0	21.0	21.0	21.0	30.0	30.0	30.0	30.0
Dicalcium phosphate	19.8	19.9	19.2	19.3	17.5	17.6	16.9	17.0	15.6	15.7	15.0	15.1
Calcium Co_3	11.7	11.7	11.7	11.7	10.9	10.9	10.9	10.8	10.2	10.1	10.1	10.0
Common salt	1.6	1.5	2.7	2.6	1.1	1.0	2.4	2.3	1.1	1.0	2.6	2.5
Sodium bicarbonate	2.0	2.1	0.3	0.5	2.7	2.8	0.8	0.9	2.6	2.8	0.4	0.6
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.4	2.3	2.7	2.6	1.9	1.9	2.2	2.2	1.8	1.8	2.1	2.1
L-Lysine HCl	5.3	4.8	4.4	3.9	4.7	4.2	3.6	3.1	4.4	4.0	3.2	2.7
L-Threonine	2.2	2.1	2.1	2.0	1.8	1.6	1.6	1.5	1.5	1.5	1.4	1.3

[1]Provided per kilogram of diet: trans-retinol, 9,000 IU; cholecalciferol, 2,500 IU; α-tocopherol acetate, 45 mg; vitamin K, 5 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; nicotineamide, 30 mg; pantothenic acid, 15 mg; folic acid, 1.1 mg; biotin, 0.13 mg; and choline, 450 mg.

[2]Provided per kilogram of diet: Mn, 100 mg; Fe, 80 mg; Zn, 100 mg; Cu, 10 mg; I, 0.5 mg; Co, 0.2 mg; Se, 0.15 mg.

lysophosphatidic acid, lysophosphatidyl inositol, and lysophosphatidyl ethanolamine) as well as polyethylene glycol ricinoleate. The hydrophilic-lipophilic balance of Artefier is 8 to 16. Based on the findings of previous research (Boontiam et al., 2017; Nemati et al., 2021), as well as the conditions of this experiment involving low-ME and low-CP diets, the doses of EB supplements that were used in this experiment were 0, 1, and 2 g/kg diet.

Growth Performance and Sample Collection

For each experimental group, BW and feed consumption were recorded on days 0, 10, 24, and 42, and after

Table 2. Calculated and analyses nutrient contents of the experimental diets with different levels of metabolizable energy (ME) and crude protein (CP) at any stage of growth (on as-fed basis).

	0−10 d				10−24 d				24−42 d			
ME level	Normal	Normal	Low	Low	Normal	Normal	Low	Low	Normal	Normal	Low	Low
CP level	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low
Calculated analysis (g/kg unless stated otherwise)												
ME (Kcal/kg)	3,000	3,000	2,900	2,900	3,100	3,100	3,000	3,000	3,200	3,200	3,100	3,100
Crude protein	230.0	218.5	230.0	218.5	215.0	204.0	215.0	204.0	195.0	185.0	195.0	185.0
Calcium	9.6	9.6	9.6	9.6	8.7	8.7	8.7	8.7	7.9	7.9	7.9	7.9
Available phosphorus	4.8	4.8	4.8	4.8	4.35	4.35	4.35	4.35	3.95	3.95	3.95	3.95
Digestible lysine	12.8	12.2	12.8	12.2	11.5	10.9	11.5	10.9	10.3	9.8	10.3	9.8
Digestible TSAA[1]	9.5	9.0	9.5	9.0	8.7	8.3	8.7	8.3	8.0	7.6	8.0	7.6
Digestible threonine	8.6	8.2	8.6	8.2	7.7	7.3	7.7	7.3	6.9	6.6	6.9	6.6
Digestible valine	9.3	8.8	9.3	8.9	8.7	8.3	8.7	8.3	7.9	7.5	7.9	7.6
Digestible tryptophan	2.0	1.9	2.1	2.0	1.8	1.7	2.0	1.9	1.6	1.5	1.8	1.7
DEB[2], mEq/kg	250	250	250	250	240	240	240	240	220	220	220	220
Analysis values[3] (g/kg unless stated otherwise)												
Crude protein	227.2	214.6	226.8	214.3	210.7	199.3	208.0	198.2	191.3	180.8	190.9	181.0
Crude fat	40.1	40.4	39.1	39.5	51.9	52.4	50.9	51.4	61.4	62.0	60.2	60.6
Total lysine	14.3	13.6	14.5	13.7	13.4	12.7	13.3	12.7	11.5	11.0	11.5	10.9
Total TSAA	10.7	10.3	10.6	10.3	9.8	9.3	9.9	9.4	9.2	8.7	9.0	8.6
Total threonine	10.3	9.8	10.2	9.7	9.5	9.0	9.4	8.8	8.3	7.9	8.3	7.8
Calcium	9.3	9.3	9.2	9.2	8.5	8.4	8.5	8.4	7.8	7.8	7.7	7.9
Total phosphorus	7.4	7.3	7.2	7.3	6.6	6.7	6.5	6.5	6.1	6.1	5.9	6.0
Gross energy, Kcal/kg	4,320	4,317	4,215	4,212	4,462	4,458	4,348	4,345	4,584	4,580	4,471	4,466
Fatty acid composition (% of total fatty acids)												
16:0	11.12	11.10	11.13	11.09	10.93	10.92	10.90	10.89	10.79	10.78	10.76	10.75
18:0	2.89	2.85	2.91	2.90	3.11	3.10	3.16	3.15	3.27	3.26	3.32	3.31
18:1n−9	22.79	22.89	22.78	22.79	22.83	22.84	22.74	22.75	22.80	22.81	22.72	22.73
18:2n−6	57.31	57.42	57.28	57.34	56.79	56.84	56.70	56.75	56.43	56.47	56.34	56.39
18:3n−3	4.00	3.84	4.04	3.99	4.46	4.42	4.60	4.56	4.82	4.79	4.96	4.92
Other	1.88	1.90	1.86	1.89	1.89	1.89	1.90	1.90	1.90	1.90	1.91	1.91

[1]Total sulfur amino acid.

[2]DEB (dietary electrolyte balance) = (Na+, mEq/kg + K+, mEq/kg) − CL−, mEq/kg.

[3]Mean of two samples per diet (Evonik Industries, Evonik Degussa GmbH, Hanau-Wolfgang, Germany).

that, the average daily gain (**ADG**) and average daily feed intake (**ADFI**) were calculated. Daily mortality incidences were collected to calculate the mortality rate. The mortality-adjusted feed conversion ratio (FCR) for each feeding period was calculated using the total ADG and total ADFI for each pen, taking into account the BW of any died or culled birds. The European performance index (**EPI**) of each experimental group was determined from d 0 to 42 using the following formula:

$$\text{EPI} = \text{livability}(\%) \times \text{liveweight}(\text{kg}) \times 100/\text{age}(\text{d}) \times \text{FCR}$$

On day 42, 2 birds from each replicate (10 birds per treatment) with a BW close to the pen mean were randomly chosen and killed by cutting the jugular vein. The customary edible sections of thighs and breasts were removed, weighed separately, and then ground without skin. The raw breast and thigh meat from each bird were packaged in plastic bags and kept at −20°C before being used for the fatty acid composition determinations. For the morphological study, 2-cm slices were cut from the central portions of each duodenum and jejunum segment, washed with distilled water to remove any remaining contents, and fixed in 10% neutral-buffered formalin.

In order to determine in vivo nutrient digestibility, a total of 120 birds (2 birds per replicate pen) were randomly chosen on day 42 to be included in a digestive trial. The acid-insoluble ash (**AIA**) marker technique was used to measure nutrient digestibility throughout the 4-d study. On day 42 (4 d before excreta collection), an additional source of AIA was supplied by the addition of Celite (Celite*545, Merck KGaA, Darmstadt, Germany) to the diet at a dosage of 10 g/kg. Ileal digesta (gut contents between Meckel's diverticulum and approximately 10 mm above the ileal−cecal junction) were collected in plastic zip bags after killing birds by cervical dislocation. The ileal digesta of two birds were pooled, and a representative sample was immediately frozen at −20°C for further determination of nutrient digestibility and apparent metabolizable energy corrected for nitrogen balance (**AMEn**).

Nutrient Digestibility

The samples from diets and ileal digesta were ground into a fine powder for chemical analysis after drying in an oven at 65°C for 24 h. Following that, the AOAC methods (2005) were used to measure the contents of dry matter (method 930.15), crude protein (N × 6.25; method 984.13), crude fat (method 920.39), and ash (method 942.05) in the feed and ileal samples. The gross energy of the sample was also measured using an automatic adiabatic oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). The content of AIA present in the feed and ileal samples was determined according to McCarthy et al. (1974). The apparent ileal digestibility (**AID**) of nutrients in diets was calculated using the following equation:

$$\text{AID}\ (\%) = [1 - (\text{AIA}_{\text{diet}}/\text{AIA}_{\text{id}}) \times (\text{Nutr}_{\text{id}}/\text{Nutr}_{\text{diet}})] \times 100$$

Where AIA_{diet} and $\text{Nutr}_{\text{diet}}$ represent the contents of AIA and nutrients in the diet (%), while AIA_{id} and Nutr_{id} reflect the contents of the same AIA and nutrients in the ileal digesta (%).

According to Majdolhosseini et al. (2019), the following equation was used to calculate the AMEn value:

$$\text{AMEn}\ (\text{Kcal/kgofdiet}) = \text{GE}_{\text{diet}} - [(\text{GE}_{\text{id}} \times \text{IF}) + 8.22 \times (\text{N}_{\text{diet}} - \text{N}_{\text{id}} \times \text{IF})]$$

Where GE_{diet} is gross energy value in the diet (Kcal/kg) and GE_{id} is gross energy value in excreta (Kcal/kg), IF is the indigestibility factor ($\text{AIA}_{\text{diet}}/\text{AIA}_{\text{id}}$), N diet is nitrogen concentration in the diet (%), N id is nitrogen concentration in excreta (%), and 8.22 is the energy equivalent (Cal/g) of uric acid.

Gut Morphology

Each intestinal tissue segment was cut into 5-mm cross-sections with a microtome and put on a glass slide before being examined using a light microscope (Olympus CX31, Shinjuku) for analysis. Three cross-sections were obtained, with each cross-section including 10 measurements. The morphological measurements of villus height (**VH**; from the base to the top of the villi), villus width (**VW**; at the mid-point of the villus), and crypt depth (**CD**; between the crypt-villus junction and the base of the crypt) were made using image-analysis software (QWinPlus v. 3.1.0; Leica Cambridge Ltd., Cambridge, UK). The VH:CD ratio was determined by combining VH and CD data. Additionally, the following equation was used to calculate the villus surface area (**VSA**): $2\pi \times (\text{VW}/2) \times \text{VH}$.

Muscle Fatty Acid Composition Total lipids were extracted from food and meat (without skin) samples using a chloroform:methanol (2:1, vol:vol) combination and quantified gravimetrically according to Folch et al. (1957). Fatty acid methyl esters were produced from total lipid in both the feed and the meat using an acid-catalyzed transesterification process described by Metcalfe et al. (1966), in which boron trifluoride was utilized, and their concentrations were determined using gas chromatography (Unicam 4600, SB Analytical, Cambridge, UK; equipped with a BPX70 fused silica capillary column and a flame ionization detector). This experiment was carried out using helium as the carrier gas, with a sample volume of 0.2 μL being injected into the column. As an internal standard, pentadecanoic acid (Sigma, St. Louis, MO) was employed. The following temperatures were programmed into the oven: 50°C for 10 min; 50°C to 180°C at 10°C/min; 180°C for 2 min; and 180°C to 240°C at 5°C/min. The temperature of the injector and detector was 280°C. The fatty acids were identified by comparing the retention times of the fatty acids to those of their respective standards. Calculations of fatty acid compositions were carried out using the

internal standard method, which was detailed by Luo et al. (2009). The total proportion of saturated fatty acids (**SFA**) was calculated as the weighted percentage sum of myristic (14:0), palmitic (16:0), and stearic (18:0) acids. The total proportions of monounsaturated fatty acids (**MUFA**) included palmitoleic (16:1c), oleic (18:1c9), and gadoleic (20:1c11). The total percentage of n−6 polyunsaturated fatty acids (**n−6 PUFA**) were determined using linoleic (18:2n6) and arachidonic (20:4n6), while total n−3 polyunsaturated fatty acids (**n−3 PUFA**) included α−linolenic (18:3n3), eicosapentaenoic (20:5n3), and docosahexaenoic (20:6n3) (22:6n3). Additionally, the total percentage of polyunsaturated fatty acids (PUFA) included both n−6 PUFA and n−3 PUFA. The ratios of PUFA to SFA as well as n−6 to n−3 PUFA were also calculated.

Statistical Analysis

The GLM procedure of SAS (SAS Institute Inc., 2010) was used to analyze the data for a 2 × 2 × 3 factorial arrangement of treatments. The 3 factors were dietary ME levels (normal vs. low), dietary CP levels (normal vs. low), and dietary EB levels (0, 1, and 2 g/kg). The applied mathematical model was as follows:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + e_{ijkl}$$

Where; Y_{ijkl} = observation, μ = overall average, A_i = effect of ME value; B_j = effect of CP level; C_k = effect of EB dose, $(AB)_{ij}$ = interaction effect of ith ME value × jth CP level; $(AC)_{ik}$ = interaction effect of ith ME value × kth EB dose; $(BC)_{jk}$ = interaction effect of jth CP level × kth EB dose; $(ABC)_{ijk}$ = interaction effect of ith ME value × jth CP level × kth EB dose; e_{ijkl} = error associated with each observation. Data on growth performance metrics were studied on a pen basis, whilst data on nutrient digestibility, intestinal morphology, and tissue fatty acid composition were analyzed on an individual bird basis. The Shapiro-Wilk and Levene tests were used to evaluate the normality and homogeneity of variances in the data, respectively. In addition, orthogonal comparisons using polynomial regression were conducted to evaluate the linear and quadratic

Table 3. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on body weight and average daily gain of broiler chickens at any stage of growth.

			Body weight (g)			Average daily gain (g/bird/d)			
ME	CP	EB (g/kg)	10 d	24 d	42 d	0−10 d	10−24 d	24−42 d	0−42 d
Normal	Normal	0	230.5	923.2	2,549^{bc}	18.87	49.48	90.33	59.70^{abc}
Normal	Normal	1	235.9	945.5	2,560^{ab}	19.37	50.69	89.69	59.95^{ab}
Normal	Normal	2	239.9	955.1	2,603^{a}	19.75	51.08	91.53	60.96^{a}
Normal	Low	0	220.4	897.8	2,452^{e}	17.86	48.38	86.36	57.39^{e}
Normal	Low	1	226.4	930.5	2,510^{bcd}	18.40	50.29	87.77	58.76^{bcd}
Normal	Low	2	239.1	931.3	2,534^{bc}	19.72	49.44	89.03	59.33^{bc}
Low	Normal	0	231.2	913.7	2,473^{de}	18.91	48.75	86.61	57.87^{de}
Low	Normal	1	233.5	935.5	2,541^{bc}	19.18	50.14	89.21	59.51^{bc}
Low	Normal	2	238.0	937.4	2,560^{ab}	19.59	49.96	90.16	59.96^{ab}
Low	Low	0	213.9	877.1	2,382^{f}	17.23	47.37	83.61	55.73^{f}
Low	Low	1	220.5	874.5	2,384^{f}	17.90	46.71	83.84	55.76^{f}
Low	Low	2	225.5	918.1	2,495^{bcd}	18.37	49.47	87.61	58.41^{cde}
SEM			6.24	10.85	17.28	0.626	0.906	1.09	0.411
Main effect means									
ME level									
Normal			232.1	930.6^{a}	2535^{a}	19.00	49.89^{a}	89.12^{a}	59.35^{a}
Low			227.1	909.4^{b}	2472^{b}	18.53	48.54^{b}	86.84^{b}	57.87^{b}
SEM			2.54	4.43	7.05	0.255	0.369	0.445	0.168
CP level									
Normal			234.8^{a}	935.1^{a}	2548^{a}	19.28^{a}	50.02^{a}	89.59^{a}	59.66^{a}
Low			224.3^{b}	904.9^{b}	2460^{b}	18.25^{b}	48.61^{b}	86.37^{b}	57.56^{b}
SEM			2.54	4.43	7.05	0.255	0.322	0.445	0.168
EB (g/kg)									
0			224.0^{b}	903.0^{b}	2464^{c}	18.22^{b}	48.50	86.73^{b}	57.67^{c}
1			229.1^{ab}	921.5^{ab}	2499^{b}	18.71^{ab}	49.46	87.63^{b}	58.50^{b}
2			235.6^{a}	935.5^{a}	2548^{a}	19.36^{a}	49.99	89.58^{a}	59.66^{a}
SEM			3.11	5.42	8.64	0.313	0.453	0.545	0.206
Significance									
ME level			0.174	0.001	<0.001	0.205	0.031	<0.001	<0.001
CP level			0.005	<0.001	<0.001	0.006	0.009	<0.001	<0.001
EB			0.038	<0.001	<0.001	0.041	0.071	0.002	<0.001
ME × CP			0.306	0.167	0.108	0.328	0.494	0.507	0.112
ME × EB			0.850	0.416	0.314	0.844	0.465	0.493	0.317
CP × EB			0.721	0.564	0.309	0.723	0.783	0.735	0.302
ME × CP × EB			0.901	0.250	0.035	0.868	0.261	0.332	0.036
Contrast effect of EB									
Linear			0.011	<0.001	<0.001	0.012	0.024	0.010	<0.001
Quadratic			0.847	0.732	0.498	0.835	0.696	0.434	0.496

$^{a-f}$Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (20 broilers/pen).

effects of increasing the dietary concentration of EB. The total mortality rate was calculated for each pen, and the data were analyzed using chi-square tests. With a $P < 0.05$ significance level, Tukey's post-hoc analysis was used to separate the means. The results are provided as mean values with their respective standard errors.

RESULTS

Growth Parameters

The performance of broiler chickens fed the experimental diets is shown in Tables 3, 4, and 5. The interactions between ME, CP, and EB were observed for final BW ($P = 0.035$), overall ADG ($P = 0.036$), and FCR ($P = 0.043$), showing that the influence of EB on these responses was greater at lower ME and CP intakes. The 2-way ME × EB interactions were also detected for ADFI at 0 to 10 d period ($P = 0.010$) and FCR at 0 to 42 d period ($P = 0.047$), indicating that the effect of EB on these responses was more marked in birds fed on low-ME. Regarding the main effect of dietary ME content, BW at 24 and 42 d, ADG in the grower, finisher, and entire experimental periods, and EPI for the entire experiment were lower ($P < 0.05$) in broiler chickens fed low-ME diets than in those fed normal-ME diets. The results also showed that ADFI and FCR increased ($P < 0.001$) as dietary ME decreased during the various experimental periods. Broiler chickens fed normal-CP diets had greater ($P < 0.05$) BW and ADG than those fed low-ME diets during the various experimental periods. The ADFI of broiler chickens receiving the low-CP diets was also lower ($P < 0.001$) in the grower, finisher, and overall experimental periods. During the starter period, the FCR of broiler chickens fed low-CP diets increased ($P = 0.002$) when compared to birds fed a high-CP diet. Additionally, the low-CP diet decreased EPI over the course of the experimental period ($P < 0.001$). Dietary EB also linearly ($P < 0.05$) increased BW, ADG, and EPI, but linearly decreased FCR over the 42-d production period.

Nutrient Digestibility Data on the AID of nutrients, as well as AMEn values, are shown in Table 6. As presented, the 3-way interaction effect of ME × CP × EB and 2-way interaction effects of ME × CP and CP × EB were not significant for any digestibility coefficient or AMEn value. In contrast, ME × EB interactions were

Table 4. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on average daily feed intake and feed conversion ratio of broiler chickens at any stage of growth.

			Average daily feed intake (g/bird/d)				Feed conversion ratio			
ME	CP	EB (g/kg)	0–10d	10–24 d	24–42 d	0–42d	0–10d	10–24 d	24–42 d	0–42d
Normal	Normal	0	23.89	78.63	187.8	112.4	1.27	1.59	2.08	1.88^{cde}
Normal	Normal	1	24.34	78.42	186.2	111.7	1.26	1.55	2.08	1.86^{def}
Normal	Normal	2	25.07	77.04	186.4	111.5	1.27	1.51	2.04	1.83^{f}
Normal	Low	0	24.12	76.57	182.9	109.6	1.35	1.58	2.12	1.91^{bcd}
Normal	Low	1	24.62	74.86	182.3	109.0	1.35	1.49	2.08	1.85^{ef}
Normal	Low	2	25.03	75.66	181.4	108.9	1.27	1.53	2.04	1.84^{ef}
Low	Normal	0	26.51	84.58	193.3	117.0	1.41	1.72	2.23	2.02^{a}
Low	Normal	1	25.65	82.91	193.4	116.6	1.34	1.66	2.17	1.96^{b}
Low	Normal	2	25.28	81.24	192.6	115.6	1.30	1.63	2.14	1.93^{bc}
Low	Low	0	26.31	79.62	189.3	113.9	1.53	1.68	2.27	2.05^{a}
Low	Low	1	26.23	78.15	188.9	113.3	1.47	1.68	2.25	2.03^{a}
Low	Low	2	24.99	76.86	185.6	111.1	1.37	1.56	2.12	1.90^{bcd}
SEM			0.514	1.16	1.62	0.858	0.043	0.036	0.029	0.017
Main effect means										
ME level										
Normal			24.51^{b}	76.86^{b}	184.5^{b}	110.5^{b}	1.30^{b}	1.54^{b}	2.07^{b}	1.86^{b}
Low			25.83^{a}	80.39^{a}	190.5^{a}	114.6^{a}	1.40^{a}	1.65^{a}	2.20^{a}	1.98^{b}
SEM			0.210	0.473	0.661	0.350	0.017	0.015	0.012	0.007
CP level										
Normal			25.13	80.30^{a}	189.9^{a}	114.2^{a}	1.31^{b}	1.61	2.12	1.91
Low			25.21	76.95^{b}	185.1^{b}	111.0^{b}	1.39^{a}	1.59	2.15	1.93
SEM			0.210	0.473	0.661	0.350	0.017	0.015	0.012	0.007
EB (g/kg)										
0			25.21	79.60	188.3	113.2	1.39^{a}	1.64^{a}	2.17^{a}	1.97^{a}
1			25.21	78.58	187.7	112.6	1.35^{ab}	1.59^{ab}	2.14^{a}	1.93^{b}
2			25.09	77.70	186.5	111.8	1.30^{b}	1.56^{b}	2.08^{b}	1.87^{c}
SEM			0.257	0.580	0.810	0.429	0.021	0.018	0.014	0.008
Significance										
ME level			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CP level			0.780	<0.001	<0.001	<0.001	0.002	0.301	0.142	0.138
EB			0.937	0.078	0.273	0.066	0.018	0.004	<0.001	<0.001
ME × CP			0.832	0.135	0.776	0.347	0.301	0.713	0.546	0.454
ME × EB			0.010	0.682	0.762	0.428	0.283	0.324	0.334	0.047
CP × EB			0.702	0.692	0.692	0.849	0.414	0.982	0.420	0.182
ME × CP × EB			0.882	0.859	0.800	0.769	0.970	0.251	0.421	0.043
Contrast effect of EB										
Linear			0.774	0.024	0.115	0.021	0.005	0.001	<0.001	<0.001
Quadratic			0.831	0.925	0.754	0.810	0.745	0.726	0.365	0.413

[a-f]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (20 broilers/pen).

Table 5. Effects of different levels of metabolisable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on European performance index (EPI) and mortality rate of broiler chickens at 0 to 42 d of age.

ME	CP	EB (g/kg)	EPI (0−42 d)	Mortality rate (0−42 d)
Normal	Normal	0	311.8	3.33
Normal	Normal	1	313.4	4.17
Normal	Normal	2	327.6	3.33
Normal	Low	0	290.3	5.00
Normal	Low	1	311.9	3.33
Normal	Low	2	314.8	4.17
Low	Normal	0	279.1	4.17
Low	Normal	1	298.8	3.33
Low	Normal	2	310.7	1.67
Low	Low	0	263.4	5.00
Low	Low	1	267.7	4.17
Low	Low	2	307.5	1.67
SEM			6.24	1.502
Main effect means				
ME level				
Normal			311.6[a]	3.89
Low			287.9[b]	3.33
SEM			2.55	0.613
CP level				
Normal			306.9[a]	3.33
Low			292.6[b]	3.89
SEM			2.55	0.613
EB (g/kg)				
0			286.2[c]	4.38
1			297.9[b]	3.75
2			315.2[a]	2.71
SEM			3.12	0.751
Significance				
ME level			<0.001	0.524
CP level			<0.001	0.525
EB			<0.001	0.293
ME × CP			0.512	1.000
ME × EB			0.083	0.457
CP × EB			0.457	0.836
ME × CP × EB			0.061	0.794
Contrast effect of EB				
Linear			<0.001	0.123
Quadratic			0.480	0.821

[a-c]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (20 broilers/pen).

observed for the AID of dry matter ($P = 0.061$), crude protein ($P = 0.048$), crude fat ($P = 0.033$), and energy ($P = 0.093$), as well as AMEn value ($P = 0.001$), indicating that supplemental EB was more effective in improving these parameters in chickens fed low-ME diets than in those fed normal-ME diets. The ME content of the diet had no effect on the AID of crude protein, crude fat, or crude ash; however, AMEn and the AID of dry matter and energy were greater in chickens fed normal-ME diets than in those fed low-ME diets. A decrease in the AID of crude protein ($P = 0.049$) and a tendency to decrease AMEn ($P = 0.092$) were observed in birds fed on low-CP diets. Inclusion of EB also linearly increased the AID of dry matter ($P = 0.018$), crude protein ($P = 0.010$), crude fat ($P = 0.004$), and energy ($P = 0.011$), as well as the AMEn content ($P < 0.001$).

Intestinal Morphology

Data on morphological parameters of the duodenum and jejunum are shown in Tables 7 and 8. As presented, the three-way interaction effect of ME × CP × EB and the two-way interaction effects of ME × CP and ME × EB were not significant for morphological indicators. In contrast, CP × EB interactions were observed for duodenal VH ($P = 0.025$) and VH/CD ratio ($P = 0.031$), indicating that supplemental EB was more effective in improving these parameters in chickens fed low-CP diets than in chickens fed normal-CP diets. The morphological parameters in both the duodenum and jejunum were not affected by the content of ME in the diet ($P > 0.05$). However, broiler chickens fed normal-CP diets had greater duodenal VH ($P = 0.005$), VH/CD ratio ($P = 0.038$), and VSA ($P = 0.060$), as well as jejunal VH ($P = 0.022$) and VSA ($P = 0.092$), compared to chickens fed low-CP diets. Inclusion of EB also linearly ($P < 0.05$) increased the VH, VH/CD ratio, and VSA in the duodenum. A decrease in the duodenal CD (linear; $P = 0.013$) was also observed in birds fed on EB-supplemented diets. Dietary supplementation with EB also tended to increase the VH ($P = 0.060$) and VSA ($P = 0.085$) in the jejunum.

Fatty Acid Profile of Breast and Thigh Meat

The fatty acid composition of both breast and thigh meat is shown in Tables 9 and 10. The main effects of dietary ME and CP content, 2-way interaction effects of ME × EB, CP × EB, and the 3-way interaction of ME × CP × EB on the fatty acid composition of breast and thigh meat were not significant. In contrast, ME × CP interactions were observed for the n−3 PUFA concentration ($P = 0.052$) and n−6 PUFA/n−3 PUFA ratio ($P = 0.037$) in the thigh meat. As the concentrations of EB inclusion in the diets increased, the SFA concentration in both breast and thigh meat increased linearly ($P < 0.05$), while the total PUFA concentration and the PUFA/SFA ratio decreased linearly ($P < 0.05$). Furthermore, the concentration of n−6 PUFA in the breast meat and the concentration of n−3 PUFA in the thigh meat dropped linearly as the level of EB supplementation increased ($P < 0.05$).

DISCUSSION

The most important finding of the current study was a significant 3-way interaction between dietary ME, CP, and EB on ADG and FCR. This suggests that using a supplementary EB is beneficial in increasing the bioavailability of energy and protein in broiler diets with a low nutrient density, which is important for boosting productive performance. In the presence of a high dietary EB level (2 g/kg), the productive performance of broilers fed a diet with low energy (100 kcal less than commercially recommended levels) and low CP and LAA (95% of the commercially recommended levels) was not significantly different from that of broilers fed a diet with recommended levels of energy, CP, and LAA. There is now a great deal of interest in the use of emulsifier supplementation to increase total fat digestibility and other nutrient utilization in broiler diets (Siyal et

Table 6. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on apparent ileal digestibility (AID) of nutrients and nitrogen-corrected apparent metabolizable energy (AME_n) in broiler chickens at 38 d of age.

ME	CP	EM (g/kg)	Dry matter %	Crude protein %	Crude fat %	Ash %	Energy %	AME_n Kcal/kg
Normal	Normal	0	76.97	71.74	81.73	51.89	73.97	3,182
Normal	Normal	1	76.83	71.84	81.99	52.24	74.20	3,195
Normal	Normal	2	77.08	71.88	82.18	53.17	74.36	3,202
Normal	Low	0	76.01	70.83	81.59	50.59	73.81	3,178
Normal	Low	1	76.05	70.99	81.79	51.22	73.99	3,183
Normal	Low	2	76.14	71.09	82.03	52.92	74.08	3,196
Low	Normal	0	73.85	70.44	79.09	51.74	71.16	3,064
Low	Normal	1	76.21	72.80	82.59	52.84	73.21	3,113
Low	Normal	2	76.66	72.92	82.88	53.99	73.60	3,153
Low	Low	0	73.32	69.45	79.26	51.22	71.24	3,048
Low	Low	1	75.66	71.85	82.43	53.17	73.65	3,106
Low	Low	2	76.30	72.18	82.61	52.92	74.21	3,140
SEM			0.876	0.748	0.939	1.14	0.812	9.7
Main effect means								
ME level								
Normal			76.51[a]	71.39	81.88	52.01	74.07[a]	3,189[a]
Low			75.33[b]	71.61	81.48	52.65	72.85[b]	3,104[b]
SEM			0.357	0.305	0.383	0.468	0.331	3.9
CP level								
Normal			76.26	71.94[a]	81.74	52.65	73.42	3,152
Low			75.58	71.07[b]	81.62	52.01	73.50	3,142
SEM			0.357	0.305	0.383	0.468	0.331	3.9
EB (g/kg)								
0			75.04[b]	70.61[b]	80.42[b]	51.36	72.55[b]	3,118[c]
1			76.19[ab]	71.87[ab]	82.20[a]	52.37	73.76[ab]	3,149[b]
2			76.54[a]	72.02[a]	82.42[a]	53.25	74.06[a]	3,173[a]
SEM			0.438	0.374	0.469	0.574	0.406	4.8
Significance								
ME level			0.024	0.625	0.456	0.339	0.012	<0.001
CP level			0.183	0.049	0.819	0.340	0.863	0.092
EB			0.048	0.019	0.007	0.076	0.027	<0.001
ME × CP			0.689	0.969	0.942	0.739	0.533	0.700
ME × EB			0.061	0.048	0.033	0.791	0.093	<0.001
CP × EB			0.996	0.984	0.982	0.940	0.983	0.998
ME × CP × EB			0.990	0.997	0.987	0.786	0.961	0.823
Contrast effect of EB								
Linear			0.018	0.010	0.004	0.024	0.011	<0.001
Quadratic			0.462	0.233	0.182	0.929	0.363	0.562

[a-c]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (2 broilers/pen).

al., 2017; Bontempo et al., 2018; Upadhaya et al., 2018). Recent research has demonstrated that the addition of an emulsifier blend (glycerol polyethylene glycol ricinoleate and lysophospholipids blend) at 0.5 g/kg in lower ME diets (50 kcal/kg) of growing pigs has a compensating effect on energy values, which can further support the productive performance of young pigs (Sun and Kim, 2019). Dietary emulsifiers may enhance the emulsification process, including the stabilization and clearance of the lipid droplet surface by bile salts, so that lipase can attach to the interphase (Siyal et al., 2017). The addition of an emulsifier to the diet may also help to improve the adsorption-desorption equilibrium, which is affected by amphiphilic molecules such as fats, phospholipids, and proteins (Majdolhosseini et al., 2019). As a result, the modifications caused by the exogenous emulsifier may increase the nutrient absorption across the enterocyte membrane, resulting in a greater nutrient bioavailability of the feed. In a previous study, Boontiam et al. (2019) also reported that lysophospholipid-supplemented birds with low-energy and low-nitrogenous diets met their nutrient requirements for productivity. This improvement in growth performance in response to EB supplementation is consistent with increases in the digestibility of crude fat and crude protein, as well as improved gut epithelial morphology, observed in the present study.

According to the findings of the current research, the ADFI and FCR of broilers fed low-ME diets were greater than those fed high-ME diets. Chickens have the ability to regulate their energy intake through their feed consumption (Massuquetto et al., 2020). Previous research demonstrated that broilers can adjust to diets with different densities by increasing their feed intake to meet their AMEn or AA requirements, with older broilers reacting more strongly to diets with lower AA density than younger broilers (Meloche et al., 2018). In a recent study in broilers, reduction of dietary ME (75 kcal/kg for each period) also decreased BW gain and increased FCR compared with the standard-ME group from 0 to 42 d (Wang et al., 2020). As evidenced by the increased FCR of broilers fed lower-density feed in the present study, the compensatory gain obtained by consuming more low-energy diets occurs less efficiently. The results of the current research also revealed that diets containing low CP and LAA (95% of the commercially recommended amounts) resulted in lower BW and FI in broilers. These results are consistent with those of a

Table 7. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on duodenum morphology in broiler chickens at 42 d of age.

ME	CP	EB (g/kg)	Villus height μm	Villus width μm	Crypt depth μm	VH/CD[1]	VSA[2] mm^2
Normal	Normal	0	1,857	124.8	164.8	11.31	0.731
Normal	Normal	1	1,863	120.5	159.2	11.98	0.705
Normal	Normal	2	1,878	124.5	166.0	11.36	0.732
Normal	Low	0	1,574	119.7	175.7	9.00	0.591
Normal	Low	1	1,795	125.1	160.3	11.31	0.700
Normal	Low	2	1,838	127.4	152.1	12.15	0.732
Low	Normal	0	1,829	124.3	171.6	10.78	0.712
Low	Normal	1	1,841	131.1	165.0	11.19	0.757
Low	Normal	2	1,865	129.6	161.2	11.66	0.751
Low	Low	0	1,554	118.7	184.1	8.53	0.581
Low	Low	1	1,807	123.3	171.9	10.69	0.702
Low	Low	2	1,855	131.4	161.7	11.53	0.763
SEM			70.13	7.82	7.58	0.688	0.048
Main effect means							
ME level							
Normal			1,801	123.7	163.0	11.19	0.698
Low			1,792	126.4	169.2	10.73	0.711
SEM			28.6	3.19	3.09	0. 281	0.0193
CP level							
Normal			1,855[a]	125.8	164.6	11.38[a]	0.731
Low			1,737[b]	124.3	167.6	10.54[b]	0.678
SEM			28.6	3.19	3.09	0.281	0.0193
EB (g/kg)							
0			1,704[b]	121.9	174.0[a]	9.90[b]	0.654[b]
1			1,827[a]	125.0	164.1[ab]	11.30[a]	0.716[ab]
2			1,859[a]	128.2	160.3[b]	11.67[a]	0.745[a]
SEM			35.1	3.91	3.79	0.344	0.0238
Significance							
ME level			0.822	0.548	0.161	0.254	0.647
CP level			0.005	0.732	0.496	0.038	0.060
EB			0.007	0.524	0.037	0.001	0.029
ME × CP			0.765	0.605	0.411	0.776	0.856
ME × EB			0.962	0.857	0.822	0.855	0.793
CP × EB			0.025	0.784	0.236	0.031	0.101
ME × CP × EB			0.989	0.831	0.827	0.827	0.872
Contrast effect of EB							
Linear			0.003	0.258	0.013	<0.001	0.009
Quadratic			0.297	0.987	0.515	0.236	0.563

[1]VH/CD, villus height to crypt depth ratio.
[2]VSA, Villus surface area (mm^2) = $2\pi \times$ (Villus width/2) × VH.
[a,b]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (2 broilers/pen).

previous investigation, which found that feeding broilers with 18.8% CP diets with 7% less digestible amino acids resulted in lower BW and BWG than feeding broilers with 17% CP diets, regardless of dietary ME content (Rehman et al., 2018). Kamran et al. (2008) also found that as dietary protein decreased during the grower, finisher, and overall experimental periods, BW gain decreased linearly while FCR increased linearly. Reduced growth performance in broilers fed on low-CP diets may be due to a deficiency in dietary protein and essential amino acid supplies (Sklan and Plavnik, 2002; Aftab et al., 2006). This deficiency may be the result of reduced feed intake or dietary protein and essential amino acid deficiencies.

The current study found that the AMEn value and the AID of dry matter and energy were reduced in a low-ME diet, whereas the digestibility of fat was unaffected by dietary ME content. Because fat, in addition to being an energy source, helps to delay the passage of food through the digestive system, including fat in the diet to a specific level allows for greater nutrient absorption and digestion in the digestive tract (Ravindran et al., 2016). As a result, it appears that broilers fed a low-ME diet have poorer nutrient digestibility than those fed a normal-energy diet. The interaction effects between dietary ME level and EB supplementation demonstrated that the AID of dry matter, protein, and crude fat, as well as the AMEn, were significantly increased in a low-ME diet supplemented with EB. These findings also indicate that a higher dietary dose of EB (2 g/kg) was superior to a lower dose (1 g/kg) in terms of nutrient digestibility and AMEn when applied to low-energy diets, whereas the addition of EB to a normal-energy diet would not have an additional beneficial effect on nutrient digestibility. Previously published studies (Majdolhosseini et al., 2019; Haetinger et al., 2021; Nemati et al., 2021) indicated that emulsion increased the AMEn value and digestibility coefficients of dry matter, energy, and crude fat in various poultry species, which is consistent with the findings of the current study. Siyal et al. (2017) also found that exogenous nutritional emulsifiers, such as lysolecithin, may aid in fatty acid digestion, particularly in the presence of poorly digested lipids and high fat intake rates. On the basis of these findings, it is reasonable to assume that lysophospholipid supplementation would increase fat digestibility in

Table 8. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on jejunum morphology in broiler chickens at 42 d of age.

ME	CP	EB (g/kg)	Villus height μm	Villus width μm	Crypt depth μm	VH/CD[1]	VSA[2] mm^2
Normal	Normal	0	1,434	135.8	172.1	8.45	0.612
Normal	Normal	1	1,471	141.6	180.8	8.35	0.656
Normal	Normal	2	1,479	138.7	169.1	9.06	0.634
Normal	Low	0	1,283	133.5	178.2	7.37	0.537
Normal	Low	1	1,389	136.7	173.2	8.25	0.606
Normal	Low	2	1,457	135.8	169.9	8.74	0.626
Low	Normal	0	1,406	128.8	184.3	7.67	0.568
Low	Normal	1	1,472	144.8	180.8	8.15	0.675
Low	Normal	2	1,486	145.0	176.2	8.73	0.676
Low	Low	0	1,259	133.2	185.7	6.80	0.527
Low	Low	1	1,377	136.0	180.6	7.68	0.588
Low	Low	2	1,420	139.1	177.9	8.05	0.619
SEM			68.9	9.35	10.88	0.699	0.0532
Main effect means							
ME level							
Normal			1,419	137.0	173.9	8.37	0.612
Low			1,404	137.8	180.9	7.85	0.609
SEM			28.1	3.81	4.44	0.285	0.0217
CP level							
Normal			1,458[a]	139.1	177.2	8.40	0.637
Low			1,364[b]	135.7	177.6	7.81	0.584
SEM			28.1	3.81	4.44	0.285	0.0217
EB (g/kg)							
0			1,345	132.8	180.1	7.57	0.561
1			1,427	139.8	178.8	8.11	0.631
2			1,461	139.7	173.3	8.64	0.639
SEM			34.4	4.68	5.44	0.349	0.0266
Significance							
ME level			0.701	0.883	0.268	0.201	0.922
CP level			0.022	0.531	0.948	0.152	0.092
EB			0.060	0.493	0.647	0.107	0.084
ME × CP			0.827	0.995	0.923	0.827	0.775
ME × EB			0.978	0.816	0.921	0.956	0.833
CP × EB			0.559	0.828	0.878	0.774	0.889
ME × CP × EB			0.969	0.906	0.925	0.946	0.837
Contrast effect of EB							
Linear			0.022	0.308	0.383	0.036	0.043
Quadratic			0.569	0.544	0.748	0.998	0.343

[1]VH/CD, villus height to crypt depth ratio.
[2]VSA, Villus surface area (mm^2) = $2\pi \times$ (Villus width/2) × VH.
[a,b]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (2 broilers/pen).

low-energy diets that include supplemental fat, particularly if the extra fat is less digestible. The current study also found that reducing dietary CP and LAA to 95% of the commercially recommended level reduced protein digestibility. This finding is consistent with the findings of a recent study conducted by Ding et al. (2016), who discovered that a 1 to 2% reduction in dietary CP significantly reduced protein digestibility in broilers while having no effect on the digestibility of dry matter and energy. The adverse effect of a low-CP diet on protein digestibility can be attributed to a dietary imbalance of energy, protein, and amino acids.

Intestinal morphology, including VH and CD values, as well as the VH/CD ratio, can be used to assess the gut health of broiler chickens (Xing et al., 2020). An increase in villi length is linked to increased epithelial turnover and cell mitosis, and a greater VH/CD ratio is linked to increased nutrient absorption (Yoon et al., 2012). Interestingly, the morphological analysis reveals two-way interactions between dietary CP levels and EB supplementation for duodenal VH and VH/CD ratio, indicating that EB was effective at mitigating the detrimental effect of a low quantity of protein and amino acids from the low-CP diet on the morphology of duodenal mucosa. However, these effects were not detected in broiler chickens receiving normal-CP diets. Previously published research demonstrated inconsistent results due to the inclusion of emulsifiers in animal diets. According to Nemati et al. (2021), supplementation with de-oiled soybean lecithin (1 and 2 g/kg) in the low-ME diet improved the VH, VH:CD ratio, and VSA in the turkey duodenum; however, only CD was decreased in the jejunum. Boontiam et al. (2017) found that dietary supplementation with lysolecithins had no effect on duodenal morphology but increased jejunal VH and VH/CD in broiler chickens. In another study, Viñado et al. (2020) reported that dietary lecithin had no significant effect on the morphological parameters of the jejunum of broilers. These contrasting results may be attributable to the fact that earlier studies used a wide range of emulsifiers with differing inclusion levels and structures, as well as different sources and rates of dietary fat. In the current investigation, the favorable effect of EB on intestinal mucosal development in 42-day-old broilers was more prominent in the duodenum than in the jejunum. One reason for this is that the duodenum is

Table 9. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on fatty acid profile (% of total fatty acids)[1] of breast meat in broilers at 42 d of age.

ME	CP	EB (g/kg)	Total SFA	Total MUFA	n−6 PUFA	n−3 PUFA	n−6:n−3PUFA	Total PUFA	PUFA:SFA
Normal	Normal	0	27.02	36.08	34.80	2.10	17.03	36.90	1.37
Normal	Normal	1	28.04	35.90	34.04	2.02	17.09	36.06	1.29
Normal	Normal	2	28.32	35.66	34.05	1.97	17.45	36.02	1.27
Normal	Low	0	27.15	36.62	34.12	2.11	16.64	36.23	1.34
Normal	Low	1	28.33	36.15	33.43	2.09	16.38	35.52	1.26
Normal	Low	2	28.51	35.82	33.73	1.94	18.56	35.67	1.25
Low	Normal	0	27.35	35.95	34.61	2.09	16.65	36.70	1.34
Low	Normal	1	28.42	35.99	33.60	1.99	17.18	35.59	1.26
Low	Normal	2	28.96	35.58	33.52	1.94	17.40	35.46	1.23
Low	Low	0	26.66	35.87	35.24	2.24	15.83	37.48	1.41
Low	Low	1	28.34	35.61	33.92	2.13	16.37	36.05	1.28
Low	Low	2	28.24	37.36	32.38	2.02	16.16	34.40	1.22
SEM			0.456	0.673	0.741	0.139	1.326	0.714	0.038
Main effect means									
ME level									
Normal			27.89	36.04	34.03	2.04	17.19	36.07	1.30
Low			27.99	36.06	33.88	2.07	16.60	35.95	1.29
SEM			0.186	0.274	0.302	0.057	0.541	0.291	0.015
CP level									
Normal			28.02	35.86	34.10	2.02	17.13	36.12	1.29
Low			27.87	36.24	33.80	2.09	16.66	35.89	1.29
SEM			0.186	0.274	0.302	0.057	0.541	0.291	0.015
EB (g/kg)									
0			27.04[b]	36.13	34.69[a]	2.14	16.54	36.83[a]	1.36[a]
1			28.28[a]	35.91	33.75[ab]	2.06	16.75	35.80[ab]	1.27[b]
2			28.51[a]	36.11	33.42[b]	1.97	17.39	35.39[b]	1.24[b]
SEM			0.228	0.336	0.370	0.069	0.663	0.357	0.019
Significance									
ME level			0.705	0.961	0.724	0.690	0.442	0.773	0.723
CP level			0.576	0.335	0.488	0.387	0.537	0.581	0.953
EB			<0.001	0.884	0.050	0.249	0.641	0.019	<0.001
ME × CP			0.190	0.876	0.590	0.503	0.536	0.489	0.219
ME × EB			0.887	0.431	0.400	0.961	0.797	0.359	0.545
CP × EB			0.795	0.539	0.770	0.919	0.927	0.717	0.848
ME × CP × EB			0.901	0.405	0.563	0.984	0.812	0.536	0.795
Contrast effect of EB									
Linear			<0.001	0.962	0.019	0.097	0.367	0.006	<0.001
Quadratic			0.076	0.623	0.502	0.953	0.796	0.491	0.130

[1]SFA (saturated fatty acids) = C14:0 + C16:0 + C18:0; MUFA (monounsaturated fatty acids) = C16:1 + C18:1 + C20:1; n-6 PUFA (n-6 polyunsaturated fatty acids) = C18:2 + C20:4; n-3 PUFA = C18:3 + C20:5 + C22:6; PUFA = n-6 PUFA + n-3 PUFA.

[a,b]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (2 broilers/pen).

where emulsified lipid droplets enter the gut and begin fat digestion (Bauer et al., 2005). The exact mode of action by which emulsifier supplements improve gut morphology is not yet fully known. There is a possibility that the emulsifier supplement, which works by stimulating micelle synthesis in the small intestine, can improve the intestinal mucosa structure of broiler chickens by decreasing intestinal fermentation and thereby minimizing villi surface damage (Mitchaothai et al., 2010; Majdolhosseini et al., 2019). Furthermore, it is hypothesized that lysophospholipids can modify the lipid bilayer of cell membranes and reduce the production of inflammatory mediators, which would improve gut integrity and support the morphological features of the gut (Chen et al., 2019; Viñado et al., 2020).

According to the results of the current study, dietary ME content had no effect on the histomorphological characteristics of the duodenal and jejunal mucosa. These findings are consistent with those of previous studies, which reported no significant variation in the gut histomorphological characteristics of broiler chickens when dietary energy was reduced by 100 kcal/kg (Wickramasuriya et al., 2019) or 150 kcal/kg (Attia et al., 2021). However, the current study found that the duodenum and jejunum of broiler chickens fed a low-CP diet showed a significant trend toward a lower VH when compared to the normal-CP group. Similarly, Allameh and Toghyani (2019) reported that a diet containing 85% of the commercially recommended levels of CP reduced the ileal VH of broiler chickens when compared to a standard-CP diet. The rate of intestinal protein synthesis appears to be decreased when a low-CP diet is fed to broiler chickens (Wykes et al., 1996). A possible explanation is that low-CP diets have lower levels of peptide-bound amino acids, resulting in decreased mucosal protein content in the small intestine (Guay et al., 2006).

The results of the current study showed that EB inclusion increased SFA content but decreased PUFA content in the breast and thigh meat, resulting in a lower PUFA:SFA ratio. However, no differences in the fatty acid composition of the breast and thigh meat were identified in response to dietary ME or CP levels. In terms of carcass quality, a decrease in the unsaturated degree of meat may be desirable in order to reduce the melting point (Sanz et al., 1999) and oxidation susceptibility

Table 10. Effects of different levels of metabolizable energy (ME), crude protein (CP), and emulsifier blend (EB) supplementation on fatty acid profile (% of total fatty acids)[1] of thigh meat in broilers at 42 d of age.

ME	CP	EB (g/kg)	Total SFA	Total MUFA	n−6 PUFA	n−3 PUFA	n−6:n−3PUFA	Total PUFA	PUFA:SFA
Normal	Normal	0	24.41	34.93	38.39	2.28	17.36	40.67	1.67
Normal	Normal	1	24.75	36.18	36.98	2.08	17.96	39.06	1.58
Normal	Normal	2	24.99	36.43	36.40	2.18	17.05	38.58	1.55
Normal	Low	0	23.82	35.73	38.36	2.10	18.63	40.46	1.70
Normal	Low	1	25.42	35.83	36.90	1.86	20.35	38.75	1.53
Normal	Low	2	25.73	36.77	35.72	1.78	20.22	37.50	1.46
Low	Normal	0	24.09	35.71	37.94	2.27	17.38	40.21	1.67
Low	Normal	1	25.21	36.49	36.41	1.88	20.03	38.30	1.52
Low	Normal	2	25.22	36.05	36.81	1.93	20.16	38.73	1.54
Low	Low	0	24.42	35.71	37.62	2.24	16.95	39.87	1.63
Low	Low	1	25.31	36.97	35.63	2.08	17.49	37.71	1.49
Low	Low	2	25.56	37.08	35.36	2.00	17.92	37.36	1.47
SEM			0.507	1.312	1.307	0.152	1.628	1.301	0.066
Main effect means									
ME level									
Normal			24.85	35.98	37.12	2.05	18.59	39.17	1.58
Low			24.97	36.33	36.63	2.07	18.33	38.70	1.56
SEM			0.207	0.535	0.533	0.062	0.664	0.531	0.026
CP level									
Normal			24.78	35.96	37.15	2.10	18.33	39.26	1.59
Low			25.04	36.35	36.60	2.01	18.59	38.61	1.55
SEM			0.207	0.535	0.533	0.062	0.664	0.531	0.026
EB (g/kg)									
0			24.18[b]	35.52	38.08	2.22[a]	17.59	40.30[a]	1.67[a]
1			25.17[a]	36.37	36.48	1.97[b]	18.96	38.46[ab]	1.53[b]
2			25.38[a]	36.58	36.07	1.97[b]	18.84	38.04[b]	1.50[b]
SEM			0.253	0.656	0.653	0.076	0.814	0.650	0.033
Significance									
ME level			0.699	0.638	0.515	0.800	0.775	0.532	0.482
CP level			0.370	0.612	0.463	0.295	0.769	0.390	0.273
EB			0.004	0.483	0.082	0.034	0.424	0.041	0.001
ME × CP			0.983	0.872	0.701	0.052	0.037	0.877	0.828
ME × EB			0.978	0.918	0.875	0.907	0.855	0.884	0.861
CP × EB			0.625	0.944	0.883	0.801	0.967	0.858	0.695
ME × CP × EB			0.531	0.888	0.990	0.734	0.683	0.998	0.868
Contrast effect of EB									
Linear			0.001	0.256	0.034	0.024	0.280	0.017	<0.001
Quadratic			0.208	0.695	0.464	0.192	0.461	0.374	0.205

[1]SFA (saturated fatty acids) = C14:0 + C16:0 + C18:0; MUFA (monounsaturated fatty acids) = C16:1 + C18:1 + C20:1; n-6 PUFA (n-6 polyunsaturated fatty acids) = C18:2 + C20:4; n-3 PUFA = C18:3 + C20:5 + C22:6; PUFA = n-6 PUFA + n-3 PUFA.

[a,b]Means within each column with no common superscript differ ($P < 0.05$). n: 5 replicate pens/treatment group (2 broilers/pen).

(Ghasemi et al., 2016) of carcass fat. Previous studies on the impact of emulsifiers on the fatty acid profile of diverse tissues have yielded contradictory results. For example, Wang et al. (2016) found no emulsifier effects on the fatty acid profile of chicken breast meat. In a study on broiler chickens, the emulsifier decreased the palmitic acid and SFA contents of the muscle; however, the UFA content, which included oleic acid, linoleic acid, and linolenic acid, did not alter significantly (Saleh et al., 2020). Additionally, Viñado et al. (2020) reported that the inclusion of soybean lecithin in broiler feed led to a decrease in the content of linoleic acid and linolenic acid in the abdominal fat pad, as well as a tendency to display a higher palmitic acid content. Supplementation with emulsifiers may facilitate the dissolution of free fatty acids that are difficult to dissolve in bile salt micelle alone, resulting in increased digestibility of SFA and, consequently, increased fatty acid accumulation in body tissue (Roy et al., 2010). This could explain why the emulsifier was able to increase the saturation degree of the breast meat in the current investigation. More research, however, is needed to better understand the mechanism of action of emulsifier supplementation on fat metabolism and fatty acid composition in different tissues.

CONCLUSIONS

In conclusion, the 3-way interaction test revealed that dietary supplementation with a multicomponent emulsifier, especially at the inclusion level of 2 g/kg, can ameliorate the detrimental effects of low-ME and low-CP diets on growth rate and feed efficiency. Furthermore, the significant interaction between ME and EB suggests that dietary EB supplementation could improve fat and protein digestibility, and thus AMEn in broiler chickens when fed a low-ME diet. Our findings also suggest that EB supplementation may be more effective in improving morphological characteristics in the duodenum than in the jejunum, particularly when applied to the diets of broilers with a lower CP content (95% of the recommendation). However, including EB in the broiler diet had an unfavorable effect on the PUFA/SFA ratio in the muscle.

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DISCLOSURES

None of the authors have any conflict of interest to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102145.

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