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EDITED BY
 Doctor Mziwenkosi Nhlanhla Mthiyane,
 North-West University, South Africa

REVIEWED BY
 Dawn A. Koltes,
 Iowa State University, United States
 Maziar Mohiti Asli,
 University of Guilan, Iran

*CORRESPONDENCE
 Whad Fayed
 ✉ whad.fayed@devenish.com
 Ahmed A. Saleh
 ✉ ahmed.saleh1@agr.kfs.edu.eg
 Mervat A. Abdel-Latif
 ✉ mervat.abdellatif@vetmed.dmu.edu.eg

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Lysophospholipid and lipase in broilers: combined lysophospholipid and lipase supplementation maintains production performance and modulates metabolic and antioxidant responses under reduced energy feeding regimens

Whad Fayed^{1,2*}, Mervat A. Abdel-Latif^{1*}, Mohammed A. Kamal³,
 Fuad Saleh², Lesley Durrans², Ahmed A. Saleh^{4*}
 and Hamada A. Ahmed¹

¹Department of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, ²Devenish Nutrition Ltd, Belfast, United Kingdom, ³Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, ⁴Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt

This study investigated the individual and synergistic effects of exogenous emulsifiers and lipase supplementation on growth performance, nutrient apparent digestibility, lipid metabolism, and gene expression in broilers receiving reduced-energy diets. All diets formulated using identical ingredient ratios except for energy-contributing components, which were adjusted through modification in soybean oil content to achieve target energy levels while maintaining amino acid profiles. Three hundred one-day-old male Ross 308 broiler chicks were randomly allocated to five treatment groups. The experiment consisted of 30 pens, with 10 birds per pen. Each treatment group included 6 replicate pens (n = 60 birds per treatment). positive control (PC) fed standard energy diets; negative control (NC) fed diets with 80 kcal/kg energy reduction; NC + emulsifier (250 g/ton Emulsifier equivalent to (20 g lysophospholipids/ton of feed); NC + lipase (100 g/ton equivalent 1 million lipase unit/ton of feed equivalent); and NC + combined emulsifier and lipase supplementation. Results demonstrated that energy reduction significantly impaired body weight gain and feed conversion ratio compared to PC birds. However, combined emulsifier and lipase supplementation restored growth performance parameters compared with NC, achieving superior body weight gain (2,222 g vs. 2,053 g in NC group) and improved feed conversion efficiency (1.344 vs. 1.491 in NC group). Crude protein retention increased significantly in emulsifier-supplemented groups (81.4–81.8% vs. 76.3% in NC), while ether extract retention improved across all supplemented treatments. Carcass characteristics remained unaffected, though abdominal fat deposition decreased significantly in all reduced-energy groups compared to PC birds. Serum biochemical analysis revealed beneficial effects on lipid metabolism, with total cholesterol and triglyceride concentrations decreasing in supplemented groups. Antioxidant status improved, evidenced by elevated glutathione peroxidase activity and reduced malondialdehyde concentration in liver tissue of treated birds. Histological examination revealed that

energy reduction caused intestinal villus atrophy and enteritis in NC birds, while emulsifier and lipase restored normal villus architecture. Gene expression analysis showed significant downregulation of insulin-like growth factor (IGF-1) mRNA in NC birds, which was restored to PC levels following combined supplementation. These findings indicate that lysophospholipid emulsifiers and exogenous lipase can effectively compensate reduced dietary energy density in broiler diets.

KEYWORDS

broiler chickens, energy density, exogenous lipase, growth performance, Lysophospholipids

Introduction

Modern broiler production demands increasingly efficient feed utilization to maintain economic viability while meeting growing global protein requirements. Dietary energy density represents a critical determinant of broiler performance, directly influencing growth rates, feed conversion efficiency, and overall production economics (Borsatti et al., 2018; Emmerson, 1997). However, rising feed costs have intensified interest in strategies that maintain optimal performance while reducing dietary energy levels.

Lipids serve as concentrated energy sources in broiler nutrition, providing approximately 2.25 times more metabolizable energy per gram than carbohydrates (Freeman, 1984; Roy et al., 2010). Despite their energy density advantages, efficient lipid utilization in young broilers faces significant physiological constraints. The immature digestive system of newly hatched chicks exhibits limited bile acid production and pancreatic lipase secretion, restricting fat digestion capacity during the critical early growth period fed on diets rich with soybean oil as a main source of fat (Krogdahl, 1985a; Noy and Sklan, 1995).

These digestive limitations have prompted extensive research into nutritional strategies that enhance lipid utilization efficiency. Bile salts naturally facilitate lipid emulsification and micelle formation, thereby improving fat digestibility and metabolizable energy values in high-lipid diets (Tanchaoenrat et al., 2013). However, the economic constraints of incorporating natural bile acids into commercial feeds have driven the development of synthetic alternatives (Al-Marzooqi and Leeson, 1999). Bile salts and their derivatives have been recently used as a dietary supplement to promote efficient growth in chickens by improving fat digestion and absorption (Alzawqaria et al., 2011).

Exogenous emulsifiers, particularly lysophospholipids, offer a cost-effective approach to enhance lipid digestibility in broiler diets. These surface-active compounds reduce interfacial tension between aqueous and lipid phases, facilitating the formation of stable emulsions that improve fat digestion and absorption (Zhao and Kim, 2017; Huang et al., 2008). Researchers demonstrated that early-life emulsifier supplementation significantly improved fat digestibility and growth performance in broiler chickens (San Tan et al., 2016; Santin et al., 2001; de Franca et al., 2023). Similarly, other reported enhanced body weight gain, feed efficiency, and lipid metabolism following emulsifier supplementation in broiler diets containing 3% soybean oil in starter diet and 3.8% and 6.8% animal fat in grower and finisher diets respectively (Bontempo et al., 2018).

Complementary to emulsifier use, exogenous lipase supplementation represents another strategy for overcoming physiological limitations in fat digestion. Pancreatic lipase deficiency during early broiler development can be compensated through dietary enzyme supplementation, potentially improving lipid hydrolysis and subsequent absorption (Adeola and Cowieson, 2011). Low-energy diets supplemented with exogenous enzymes have demonstrated positive effects on broiler growth performance, suggesting potential for maintaining production efficiency under reduced-energy feeding regimens (Francesch and Geraert, 2009).

Although emulsifiers and exogenous lipases are often evaluated in high-fat diets, their role in improving digestive efficiency under reduced-energy feeding strategies is also of practical interest. Young broilers have limited bile salt secretion and pancreatic lipase activity, which may restrict lipid digestion efficiency. Therefore, supplementation with lysophospholipids and lipase may enhance lipid utilization and help compensate for reduced dietary energy. Accordingly, this study evaluated whether their combined supplementation could maintain growth performance in broilers fed reduced-energy diets.

Recent research has yielded conflicting results regarding the efficacy of individual versus combined emulsifier and lipase supplementation strategies. While some studies report significant performance improvements with emulsifier supplementation (San Tan et al., 2016; Bontempo et al., 2018), others have found minimal effects on broiler growth when emulsifiers or lipases are used individually in low-energy diets (Arshad et al., 2020; Wickramasuriya et al., 2020). These inconsistent findings suggest that synergistic interactions between emulsifiers and lipases may be necessary to achieve optimal results in energy-restricted feeding systems.

The potential for combined supplementation strategies to compensate for reduced dietary energy density has received limited investigation. Understanding the individual and interactive effects of lysophospholipids and exogenous lipase on broiler performance, nutrient utilization, and physiological responses could provide valuable insights for developing sustainable feeding programs that maintain production efficiency while reducing feed costs.

Therefore, this study was designed to evaluate the individual and combined effects of lysophospholipid emulsifiers and exogenous lipase supplementation on growth performance, nutrient digestibility, blood biochemistry, antioxidant status, intestinal morphology, and growth-related gene expression in broiler chickens fed energy-reduced diets. The findings will contribute to the development of evidence-based nutritional management strategies for sustainable broiler production under challenging economic conditions.

Materials and methods

Experimental design and ethics

This research was conducted following the animal welfare guidelines established by Damanhour University, Egypt, with all procedures approved by the Institutional Animal Care and Use Committee (DMU/VetMed-2025/017). The study employed a completely randomized design to evaluate five dietary treatments over a 32-day experimental period.

Birds and housing management

Three hundred one-day-old Ross 308 males broiler chicks were obtained from a commercial hatchery and randomly distributed into five treatment groups of 60 birds each. The pen (10 birds per pen; 6 pens per treatment) was considered the experimental unit. Cages are metal wire-floor (110 x 90 x 50 cm³) with no litter material allowing droppings fill through wire mesh into collection trays beneath the cages.

The cages were installed in open-sided poultry facilities with concrete floors and adequate ventilation systems. Environmental conditions were carefully monitored and maintained throughout the experimental period. Ambient temperature was regulated at 33°C on one day old chicks and decreased by 2°C weekly to 25°C at age 32 days, while relative humidity was maintained at 50-70%. Lighting followed a standard commercial schedule with continuous illumination during the first week, followed by progressive reduction to achieve 18 hours of light and 6 hours of darkness at age 28 days gradually decreasing 1.5 hours of light weekly. Fresh water and feed were provided *ad libitum* throughout the study period. Cup drinkers used 3 per cage and trough feeders used.

No mortality was observed during the experimental period. Therefore, feed intake and feed conversion ratio were calculated directly based on the initial number of birds per replicate without mortality correction.

Dietary treatments and feed formulation

Five experimental Diets were formulated to closely approximate Ross 308 nutrient recommendations, with intentional ME reduction applied only to NC treatments. Birds were fed on starter diet from 0 to 10 days, grower diets from 11 to 24 days and finisher diets from 25 days to the end of experiment at 32 days. Diet compositions are presented in [Table 1](#).

Treatment groups were as follows: Positive Control (PC): Standard energy diets containing optimal metabolizable energy levels (2,975, 3,050, and 3,100 kcal/kg for starter, grower, and finisher phases, respectively). Negative Control (NC): Energy-reduced diets formulated with 80 kcal/kg lower metabolizable energy than PC diets (2,895, 2,970, and 3,020 kcal/kg for respective phases). NC + Emulsifier: Energy-reduced diets supplemented with lysophospholipid emulsifier (Smart LPL, Devenish Nutrition Ltd, Belfast, Northern Ireland, UK at 250 g/ton of feed) the product is 8% Lyso-phospholipids from hydrolyzed soybean lecithin as 80gm

lysophospholipids/kg), at 250 g/ton of feed. NC + Lipase: Energy-reduced diets supplemented with exogenous lipase enzyme (Smart Lipase, Devenish Nutrition Ltd, Belfast, Northern Ireland, UK at 100g/ton) concentration 10,000 U/g. NC + Combined: Energy-reduced diets supplemented with both lysophospholipid emulsifier (250 g/ton) and exogenous lipase (100 g/ton).

All diets were formulated using identical ingredient ratios except for energy-contributing components, which were adjusted through modification in soyabean oil content and wheat bran inclusion to achieve target energy levels while maintaining protein and amino acid profiles.

Sampling protocol

The individual birds utilized in the apparent digestibility trial (3 birds per pen; 18 birds per treatment) were designated as the experimental units for assessing carcass quality, blood biochemical parameters, antioxidant status, intestinal morphology and gene expression.

Apparent digestibility trial procedures

On day 28 of age, three birds per replicate (18 birds per treatment) selected to represent the average body weight of the replicate, were randomly transferred to individual metabolic cages for nutrient digestibility assessment. Following a 48-hour adaptation period, total excreta collection was conducted from day 30 to day 32. Following bird transfer, the remaining birds in each replicate were maintained in their original cages for continued growth performance evaluation until day 32 (final slaughter age). Feed intake and feed conversion ratio during this period were calculated based on the adjusted number of birds per replicate. The transferred birds were excluded from performance calculations from the day of removal onward. No mortality was recorded throughout the experimental period; therefore, performance calculations required adjustment only to account for the intentional removal of birds for metabolic evaluation. Fresh excreta samples were immediately collected, weighed, and dried in a forced-air oven at 60°C for 24 hours until constant weight was achieved. Dried samples were ground through a 1-mm screen using a laboratory mill and stored in sealed containers at room temperature pending chemical analysis.

Performance measurements

Individual bird weights were recorded weekly using digital scales accurate to ±1 g. Feed consumption was monitored daily by weighing feed offered and refused for each replicate pen.

Body weight gain, feed intake, and feed conversion ratio (FCR) were calculated for each experimental phase and cumulatively for the entire trial period.

Carcass traits

At 32 days of age, after completion apparent digestibility trial, the birds were weighed and humanely slaughtered by cervical dislocation following a 12-hour feed withdrawal period.

TABLE 1 Composition of experimental diets.

Ingredients	PC			NC (- 80 kcal)		
	Starter (0–10) d	Grower (11–24) d	Finisher (25–32) d	Starter (0–10) d	Grower (11–24) d	Finisher (25–32) d
Yellow Corn, Ground	56.45	62.71	66.74	56.65	61.8	65.02
Soybean Meal (46 CP %)	36.4	31.11	27.46	35.85	30.5	26.79
Soyabean Oil	1.52	1.58	1.64	0.5	0.75	1
Wheat Bran	2	2	2	3.69	4.38	5.06
Limestone	1.3	0.94	0.79	1.31	0.95	0.81
Sodium chloride	0.21	0.21	0.21	0.2	0.2	0.2
Sodium-Bicarbonate	0.23	0.22	0.22	0.23	0.23	0.23
Mono Calcium Phosphate	0.8	0.49	0.25	0.77	0.45	0.21
Choline Chloride (60%)	0.08	0.08	0.07	0.08	0.08	0.07
L-Lysine HCl (77.9%)	0.22	0.22	0.21	0.23	0.22	0.21
L-Threonine	0.08	0.07	0.06	0.08	0.07	0.05
DL-Methionine (99%)	0.36	0.32	0.3	0.36	0.32	0.3
Ant Cox (Maduramycin 1%)	0.05	0.05	0.05	0.05	0.05	0.05
Premix ¹	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100
Calculated nutrient and energy						
Dry Matter %	89.78	89.38	89.12	89.62	89.24	88.99
CP%	21.67	19.69	18.30	21.68	19.71	18.34
Fat%	4.49	4.66	4.78	3.53	3.89	4.21
ME (KCal/Kg diet)	2975	3050	3100	2895	2970	3020
Fiber%	3.08	2.98	2.91	3.24	3.19	3.18
Av Phos%	0.50	0.42	0.36	0.50	0.42	0.36
Calcium%	0.95	0.75	0.65	0.95	0.75	0.65
Sodium%	0.18	0.18	0.18	0.18	0.18	0.18
Chloride%	0.23	0.23	0.23	0.23	0.23	0.23
Lys%	1.34	1.20	1.10	1.34	1.20	1.10
Met%	0.68	0.62	0.58	0.67	0.62	0.58
Met+Cys%	1.02	0.94	0.88	1.02	0.94	0.88
Thr%	0.90	0.80	0.74	0.90	0.80	0.74
Analyzed nutrient composition %						
Dry Matter %	89.2	89.3	89.7	89.2	89	89.6
CP%	21.9	19.4	18.8	21.85	19.31	18.7
Fat%	5.1	4.7	5	3.7	4	4.5
Fiber%	3	3	3.1	2.9	3.3	3.25

¹Dietary supplements and additives included: Vitamin–mineral premix (3000 mg·kg⁻¹ inclusion; Active Vet Co.): Each 1 kg of diet provided approximately: Vit. A (12,000 IU), Vit. D₃ (3,000 IU), Vit. E (7 mg), Vit. K₃ (5 mg), Vit. B₁ (5 mg), Vit. B₂ (2 mg), Vit. B₆ (6 mg), Vit. B₁₂ (30 µg), Vit. B₃ (30 mg), Vit. C (4.5 mg), choline chloride (10 mg), folic acid (3 mg), biotin (6 mg), copper (30 mg), iron (100 mg), zinc (1.8 mg), cobalt (3 mg), and magnesium (30 mg) choline chloride (1000 mg·kg⁻¹) phytase 1000 FTU/kg.

Carcass components including deboned breast muscle, Deboned thigh muscle, liver, heart, spleen, and abdominal fat pad were carefully dissected and weighed to determine relative organ weights as percentages of live body weight.

Blood sample collection and processing

Blood samples were collected from the brachial vein following 12 hours of feed removal using heparinized syringes with conc 20 IU/ml. Samples were immediately placed on ice and centrifuged at 1050 g for 20 minutes at 4°C to separate plasma. The resulting plasma was aliquoted into sterile microtubes and stored at -20°C until biochemical analysis.

Chemical analyses

Feed and excreta samples were analyzed in duplicate for proximate composition following standard AOAC International methods (AOAC, 2019). Dry matter content was determined by oven drying at 105°C for 24 hours (Method 930.15). Crude protein was analyzed using the Kjeldahl method with nitrogen-to-protein conversion factor of 6.25 (Method 968.06). Ether extract was determined by petroleum extraction using Soxhlet apparatus (Method 920.39), while crude fiber was analyzed following acid-base digestion procedures (Method 932.09).

Apparent retention coefficients were calculated using the following formula:

$$\text{Retention (\%)} = \left[\frac{(\text{Nutrient intake} - \text{Nutrient in excreta})}{\text{Nutrient intake}} \right] \times 100$$

Plasma biochemical analysis

Plasma samples were analyzed for various biochemical parameters using commercial diagnostic kits (Diamond Diagnostics, Egypt) and automated clinical chemistry analyzer following manufacturer protocols. Parameters measured included total cholesterol (CH 1210) Enzymatic colorimetric method (CHOD-POD), triglycerides (TR 2010) – Enzymatic colorimetric method (GPO-POD), high-density lipoprotein cholesterol (HDL-C) (HD 1610) – Direct enzymatic colorimetric method., low-density lipoprotein cholesterol (LDL-C) (LD 1910) – Direct enzymatic colorimetric method., glucose (GL 1310) –Enzymatic colorimetric method (GOD-POD), total protein (TP 1410) – Biuret method., albumin (AL 1010) – Bromocresol Green (BCG) method, alanine aminotransferase (ALT) (AT 1020) – Kinetic UV method following IFCC recommendations., and aspartate aminotransferase (AST) (AS 1020) – Kinetic UV method following IFCC recommendations.

Antioxidant status assessment

The individual bird from which liver samples were collected served as the experimental unit. Liver tissue samples (approximately 0.5 g) were collected from sacrificed birds and immediately frozen in liquid nitrogen before storage at -80°C. Tissue homogenates were prepared in ice-cold phosphate buffer (pH 7.4) using a high-speed homogenizer. Following centrifugation at 10,000 × g for 15 minutes at 4°C, supernatants were collected for antioxidant enzyme analysis.

Glutathione peroxidase (GPX) and catalase (CAT) activities were measured using spectrophotometric methods according to established protocols (Marklund and Marklund, 1974). Malondialdehyde (MDA) concentration, as an indicator of lipid peroxidation, was determined using the thiobarbituric acid reactive substances (TBARS) method (Richard et al., 1992).

Histological examination

Approximately 2-cm segments from the middle part of jejunum were collected from sacrificed birds and immediately fixed in 10% neutral buffered formalin for 48 hours. Fixed tissues were processed through graded alcohol series (70% to 100%) for dehydration, cleared in xylene, and embedded in paraffin wax. Tissue sections (4-5 μm thickness) were cut using a microtome and stained with hematoxylin and eosin (H&E) following standard protocols (Bancroft and Gamble, 2008).

Histological sections were examined under light microscopy at 400× magnification. Villus height measurements were performed using calibrated image analysis software (ImageJ, NIH, Bethesda, MD, USA). A minimum of 10 well-oriented villi per section were measured from each bird, with measurements taken from the villus tip to the base of the crypt.

Gene expression analysis

Liver tissue samples (approximately 50 mg) were collected from six birds per treatment group at slaughter (age 32 days) and immediately snap-frozen in liquid nitrogen before storage at -80°C. Total RNA was extracted using TRIzol™ Reagent (Invitrogen, Life Technologies, USA; Cat. #15596026) following the manufacturer's instructions. To eliminate genomic DNA contamination, on-column DNase I digestion was performed during extraction. RNA quality was assessed by 2% agarose gel electrophoresis; samples with a 28S:18S ribosomal RNA ratio < 1.8 were excluded. RNA purity was quantified using a NanoDrop spectrophotometer (Quawell Q5000, USA); only samples with A260/A280 ≥ 1.8 and A260/A230 ≥ 1.6 were used for downstream analysis.

First-strand cDNA was synthesized from 1 μg of total RNA per sample using the SensiFAST™ cDNA Synthesis Kit (Bioline, UK; Cat. #BIO-65053) according to the manufacturer's protocol, which incorporates random hexamer and oligo-dT priming. The resulting cDNA was diluted 1:10 in nuclease-free water and stored at -20°C until use. Quantitative real-time PCR (qRT-PCR) was performed in 20 μL reaction volumes consisting of 10 μL SensiFAST™ SYBR® Lo-ROX Mix (Bioline, UK; Cat. #BIO-73005; 2×), 200 nM of each primer, and 2 μL of diluted cDNA template. Reactions were run on a Stratagene MX3000P thermocycler (Agilent Technologies, USA) using the following cycling conditions: initial denaturation at 95°C for 2 min; 40 cycles of 95°C for 5 s (denaturation) and 60°C for 20 s (combined annealing and extension); followed by a continuous melt-curve stage from 60°C to 95°C (0.1°C/s ramp rate) to confirm single-product amplification. Each biological replicate (n = 6 per group) was assayed in technical triplicate; the mean quantification cycle (Cq) of the three technical replicates was used for subsequent calculations. Technical replicates with inter-run Cq standard deviations exceeding 0.5 were excluded and re-run. No-template controls (NTC) and no-

reverse-transcriptase controls (No-RT) were included on every plate; all controls yielded Cq values ≥ 35 , confirming the absence of primer-dimers and genomic DNA contamination, respectively.

Gene-specific primers for insulin-like growth factor-1 (IGF-1) and β -actin were designed using Primer3 software (Untergasser et al., 2012) based on Gallus gallus mRNA sequences retrieved from NCBI (Table 2). Primers were synthesized and HPLC-purified by a commercial supplier. Single-product amplification was verified by melt-curve analysis (single peak at the expected $T_m \pm 1^\circ\text{C}$) and agarose gel electrophoresis (2% gel; ethidium bromide staining) of representative amplicons, which yielded bands of the expected sizes: IGF-1, 198 bp; β -actin, 195 bp.

PCR amplification efficiency was determined from standard curves generated by five-point, five-fold serial dilution of a pooled cDNA standard. Efficiency was $98.6 \pm 1.2\%$ for IGF-1 ($R^2 = 0.998$) and $99.1 \pm 0.9\%$ for β -actin ($R^2 = 0.999$). Reference gene stability was confirmed by geNorm analysis (M-value < 0.5 for β -actin across all treatment groups), validating its suitability as a reference under the experimental conditions (Vandesompele et al., 2002). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (Livak and Schmittgen, 2001), with β -actin as the reference gene and the positive control group as the calibrator.

Statistical analysis

All data were analyzed using a one-way ANOVA within the General Linear Model (GLM) procedure of SPSS (Version 17.0). Dietary treatment (five levels: PC, NC, NC + emulsifier, NC + lipase, NC + emulsifier + lipase) was included as a fixed effect in the model. For each response variable, the model used was: $(Y_{ij} = \mu + T_i + e_{ij})$,

where Y_{ij} is the observed value, μ is the overall mean, T_i is the effect of the dietary treatment, and e_{ij} is the random error term. Tukey's post-hoc test was applied when the treatment effect was significant ($P < 0.05$). Each parameter was analyzed using its appropriate experimental unit with results presented as least squares means \pm standard error of the mean. For qPCR data, analyses were performed on Cq-derived quantities after efficiency correction; treatment was modeled as a fixed effect, with biological replicate as the experimental unit and technical triplicates averaged. Normality and homoscedasticity were checked on model residuals; Tukey's HSD controlled type-I error ($\alpha = 0.05$).

Results

Growth performance

Growth performance parameters measured throughout the 32-day experimental period are presented in Table 3. Initial body weight did not differ among treatment groups ($P = 0.967$), confirming successful randomization.

Energy reduction significantly affected growth performance. Birds fed the negative control (NC) diet showed lower final body weight (2,098 g) compared to the positive control (PC; 2,218 g; $P < 0.001$). Similarly, cumulative body weight gain was reduced in the NC group (2,053 g) relative to PC birds (2,172 g; $P < 0.002$).

Supplementation strategies improved growth performance. The combined emulsifier + lipase treatment produced the highest final body weight (2,268 g) and body weight gain (2,222 g), significantly exceeding both NC and PC groups ($P < 0.001$). Emulsifier

TABLE 2 Gene-specific primers used for quantitative real-time PCR.

Gene	Direction	Sequence (5'→3')	Amplicon (bp)	GenBank Accession
IGF-1	Forward	5'-TGCTTGCTCACCTTTACCAGCTC-3'	198 bp	NM_001004384.2
IGF-1	Reverse	5'-AAGTGTACTTCCTTCTGAGTCTTGAG-3'		
β -actin	Forward	5'-TGCTGTGTCCCATCTATCG-3'	195 bp	NM_205518.1
β -actin	Reverse	5'-TTGGTGACAATACCGTGTTC-3'		

IGF-1, insulin-like growth factor-1; β -actin, reference gene; bp, base pairs.

TABLE 3 Differentiation of single and combined impact of lysophospholipids and lipase on growth performance and nutrients retention in broilers.

Parameter	PC	NC	NC + emulsifier	NC + lipase	NC + emulsifier + lipase	P-Value
Initial body weight, g	45.7 \pm 0.59	45.4 \pm 0.56	45.6 \pm 0.28	45.5 \pm 0.42	45.8 \pm 0.29	0.967
Final body weight, g (32 d)	2218 \pm 13 ^b	2098 \pm 13 ^d	2208 \pm 7 ^b	2169 \pm 12 ^c	2268 \pm 8 ^a	0.001
ADG, g	67.88 \pm 1.02 ^{ab}	64.15 \pm 0.55 ^b	67.73 \pm 1.16 ^{ab}	66.36 \pm 1.32 ^{ab}	69.45 \pm 0.95 ^a	0.022
ADFI, g	96.81	97.72	96.4	96.84	95.13	0.608
FCR	1.398 \pm 0.01 ^{bc}	1.491 \pm 0.02 ^a	1.399 \pm 0.02 ^{bc}	1.431 \pm 0.03 ^{ab}	1.344 \pm 0.03 ^c	0.004
Crude protein retention, %	78.5 \pm 0.21 ^b	76.3 \pm .55 ^c	81.4 \pm 0.39 ^a	79.6 \pm 0.49 ^b	81.8 \pm 0.55 ^a	0.022
Crude fiber retention, %	26.5 \pm 1.3	25.3 \pm 1.7	25.2 \pm 2.3	25.1 \pm 0.8	25.8 \pm 0.85	0.964
Ether extract retention, %	47.2 \pm 0.6 ^a	45.7 \pm 0.7 ^b	48.1 \pm 0.7 ^a	47.2 \pm 0.9 ^a	49.3 \pm 0.3 ^a	0.018

PC, positive control; NC, negative control (-80kcal); Means in each row with different superscripts are significantly different at $P < 0.05$.

TABLE 4 Differentiation of single and combined impact of lysophospholipids and lipase on organ weights in broiler chickens in relative to live body weight at 32 days age n=18 from each treatment.

Parameter	PC	NC	NC + emulsifier	NC +lipase	NC + emulsifier + lipase	P-Value
Carcass, % to LBW ¹	72.5 ± 0.13	72.16 ± 0.18	72.40 ± 0.34	72.71 ± 0.24	72.19 ± 0.16	0.403
Deboned Breast muscle weight, %	24.29 ± 0.35	24.66 ± 0.12	24.89 ± 0.50	24.64 ± 0.31	25.13 ± 0.19	0.461
Thigh muscle weight, %	18.21 ± 0.45	17.95 ± 0.47	18.11 ± 0.37	17.98 ± 0.31	18.48 ± 0.61	0.928
Gizzard weight, %	1.01 ± 0.07 ^c	1.03 ± 0.05 ^c	1.05 ± 0.04 ^{bc}	1.23 ± 0.04 ^a	1.21 ± 0.06 ^{ab}	0.017
Liver weight, %	2.08 ± 0.10	1.98 ± 0.07	1.82 ± 0.08	2.05 ± 0.09	1.98 ± 0.08	0.252
Spleen weight, %	0.145 ± 0.03	0.127 ± 0.02	0.077 ± 0.01	0.092 ± 0.02	0.103 ± 0.01	0.185
Abdominal fat, %weight	1.87 ± 0.05 ^a	1.22 ± 0.05 ^c	1.13 ± 0.05 ^c	1.53 ± 0.06 ^b	1.34 ± 0.12 ^{bc}	0.031
Heart weight, %	0.437 ± 0.04	0.467 ± 0.02	0.505 ± 0.04	0.463 ± 0.02	0.445 ± 0.02	0.567
1: live body weight.	% means the organs weight to live body weight					

PC, positive control; NC, negative control (-80kcal); Means in each row with different superscripts are significantly different at P<0.05.

supplementation alone achieved body weight gain (2,162 g) comparable to PC birds, while lipase alone resulted in intermediate improvement (2,123 g).

Feed intake differed among treatments (P < 0.005). The combined supplementation group consumed less feed (3,044 g) than other treatments while maintaining superior growth. Consequently, feed conversion ratio (FCR) improved significantly. The combined treatment achieved the best FCR (1.344), representing a 9.9% improvement over NC (1.491; P < 0.004). Emulsifier alone restored FCR to PC levels (1.399), while lipase alone produced intermediate improvement (1.431).

Nutrient retention

Apparent retention coefficients are summarized in Table 3. Crude protein retention decreased in the NC group (76.3%) compared to PC (78.5%; P < 0.022). Emulsifier supplementation

alone (81.4%) and combined supplementation (81.8%) significantly improved protein retention.

Ether extract retention decreased in NC birds (45.7%). Supplemented groups showed improved fat retention, with the combined treatment achieving the highest value (49.3%; P < 0.018).

Crude Fiber retention was unaffected by treatment (P = 0.964).

Carcass traits and organ weights

Carcass traits and relative organ weights are presented in Table 4. Overall carcass yield was not affected by treatment (P = 0.403), ranging from 72.16% to 72.71%. Breast and thigh muscle proportions were also unaffected (P > 0.05).

Gizzard relative weight differed among treatments (P < 0.017), with lipase-supplemented groups showing higher values than controls (Saleh et al., 2025).

TABLE 5 Differentiation of single and combined impact of lysophospholipids and lipase on blood parameters in broiler chickens at 32 days age n=18 from each treatment.

Parameter	PC	NC	NC + emulsifier	NC +lipase	NC + emulsifier + lipase	P-Value
Total protein, (mg/dL)	2.67 ± 0.06	2.62 ± 0.10	2.71 ± 0.17	2.69 ± 0.09	2.68 ± 0.12	0.781
Albumin (mg/dL)	1.20 ± 0.06	1.35 ± 0.05	1.39 ± 0.09	1.48 ± 0.03	1.48 ± 0.12	0.109
Globulin (mg/dL)	1.46 ± 0.08	1.29 ± 0.07	1.31 ± 0.24	1.21 ± 0.07	1.20 ± 0.15	0.698
Total Cholesterol (mg/dL)	141.3 ± 1.8a	129.8 ± 1.5b	126.7 ± 1.7bc	128.2 ± 1.0bc	123.2 ± 2.02c	0.037
Triglycerides (mg/dL)	8.43 ± 0.28a	6.33 ± 0.23b	6.66 ± 0.17b	6.50 ± 0.26b	6.08 ± 0.22b	0.024
HDL-cholesterol (mg/dL)	86.7 ± 4.2	87.0 ± 3.1	88.7 ± 1.3	86.5 ± 1.9	88.3 ± 1.9	0.967
LDL-cholesterol (mg/dL)	37.7 ± 4.5	30.17 ± 1.9	30.3 ± 1.9	31.3 ± 2.2	31.2 ± 1.01	0.249
GOT (I/U)	287.3 ± 23	262.0 ± 6	257.7 ± 8	257.3 ± 12	259.7 ± 7	0.466
GPT (I/U)	4.11 ± 0.30a	3.83 ± 0.25b	3.37 ± 0.14b	3.30 ± 0.14b	3.29 ± 0.15b	0.032
Glucose, (mg/dL)	140.5 ± 3	140.0 ± 3	138.2 ± 3	139.3 ± 2	139.7 ± 3	0.984

PC, positive control; NC, negative control (-80kcal); Means in each row with different superscripts are significantly different at P<0.05.

TABLE 6 Differentiation of single and combined impact of lysophospholipids and lipase on lipid peroxidation in broilers n=18 from each treatment.

Parameter	PC	NC	NC + emulsifier	NC +lipase	NC + emulsifier + lipase	P-Value
GPX, U/g protein	36.21 ± 0.43b	40.04 ± 0.19a	39.17 ± 0.29a	39.04 ± 0.18a	39.29 ± 0.39a	0.002
CAT, μmol/g protein	22.54 ± 0.59	23.61 ± 0.36	23.30 ± 0.31	23.56 ± 0.08	23.59 ± 0.25	0.253
MDA, nmol/g protein	9.02 ± 0.17a	7.27 ± 0.18bc	7.01 ± 0.13bc	6.83 ± 0.19c	7.58 ± 0.34b	0.004

PC, positive control; NC, negative control (-80kcal); Means in each row with different superscripts are significantly different at $P < 0.05$.

Abdominal fat percentage differed significantly ($P < 0.031$). The PC group showed the highest fat deposition (1.87%), whereas all energy-reduced groups exhibited lower values (1.13%–1.53%).

Liver, spleen, and heart weights remained within normal physiological ranges with no significant differences among treatments.

Blood biochemistry

Plasma biochemical parameters are shown in Table 5. Total protein, albumin, and globulin concentrations were not affected by treatment ($P > 0.05$).

Total cholesterol decreased progressively with energy reduction and supplementation. PC birds showed the highest levels (141.3 mg/dL), while the combined treatment showed the lowest (123.2 mg/dL; $P < 0.037$).

Triglyceride concentrations followed a similar trend ($P < 0.024$). HDL and LDL fractions were not significantly affected ($P > 0.05$).

GOT activity decreased in supplemented groups compared to PC ($P < 0.032$), while GPT and glucose levels remained unchanged ($P > 0.05$).

Antioxidant status

Hepatic glutathione peroxidase (GPX) activity increased significantly in supplemented groups (39.04–40.04 U/g protein) compared to PC (36.21 U/g protein; $P < 0.002$). Catalase activity was unaffected ($P = 0.253$) (Table 6).

Malondialdehyde (MDA) concentrations were significantly reduced in supplemented groups (6.83–7.58 nmol/g protein) compared to PC (9.02 nmol/g protein; $P < 0.004$) (Table 6).

Intestinal morphology

Jejunal villus height differed significantly among treatments (Table 7, Figure 1). NC birds showed reduced villus height (1,489 μm) compared to PC (1,893 μm; $P < 0.001$).

Emulsifier supplementation restored villus height (1,991 μm), lipase partially improved it (1,861 μm), and the combined treatment produced the greatest height (2,040 μm).

Gene expression

Hepatic IGF-1 mRNA expression (Table 7, Figure 2) was reduced in NC birds (4.097-fold) compared to PC (5.043-fold; $P < 0.001$). Emulsifier alone (5.520-fold) and combined supplementation (5.847-fold) significantly increased IGF-1 expression above PC levels.

Discussion

The improvement of lipid digestion and nutrient utilization has been consistently associated with enhanced growth performance in broiler chickens. Dietary emulsifiers and lysophospholipids have been shown to improve body weight gain and feed conversion ratio by facilitating fat digestion and increasing nutrient absorption efficiency (Salari et al., 2024; Wang et al., 2024; Cowieson et al., 2006). Furthermore, lysophospholipid supplementation has been reported to enhance intestinal morphology and nutrient digestibility, contributing to improved feed efficiency and overall growth performance (Haetinger et al., 2021; Eid et al., 2025). These positive responses are primarily attributed to improved lipid metabolism and increased energy availability for tissue accretion (Eid et al., 2025).

The present study clearly demonstrated that combined supplementation with lysophospholipids and exogenous lipase effectively compensates for reduced dietary energy density in broiler diets. Energy restriction alone resulted in marked reductions in growth performance, nutrient retention, intestinal development, antioxidant status, and IGF-1 expression, confirming that inadequate dietary energy limits the growth potential of modern broiler strains characterized by high metabolic demands (Leeson and Summers, 2005; Zampiga et al., 2016). Interestingly, the combined supplementation strategy not only restored performance under energy-restricted conditions but also produced superior final body weight and feed conversion ratio compared with the positive control. This suggests a synergistic interaction between enhanced lipid emulsification and increased triglyceride hydrolysis capacity.

TABLE 7 Small intestine villus height (μm) and liver mRNA gene expression of IGF-1 in different experiment groups at age 32 days. Data was expressed as means ± SE. Significance was considered at ($P < 0.05$) n=18 from each treatment.

Parameter	PC	NC	NC + emulsifier	NC +lipase	NC + emulsifier + lipase	P-Value
Villus height	1892.90 ± 92.87 ^a	1489.50 ± 92.59 ^b	1990.51 ± 93.82 ^a	1861.16 ± 93.10 ^a	2039.72 ± 94.05 ^a	0.001
IGF-1	5.043 ± 0.086 ^a	4.097 ± 0.081 ^b	5.520 ± 0.218 ^a	4.933 ± 0.067 ^a	5.847 ± 0.182 ^a	<0.001

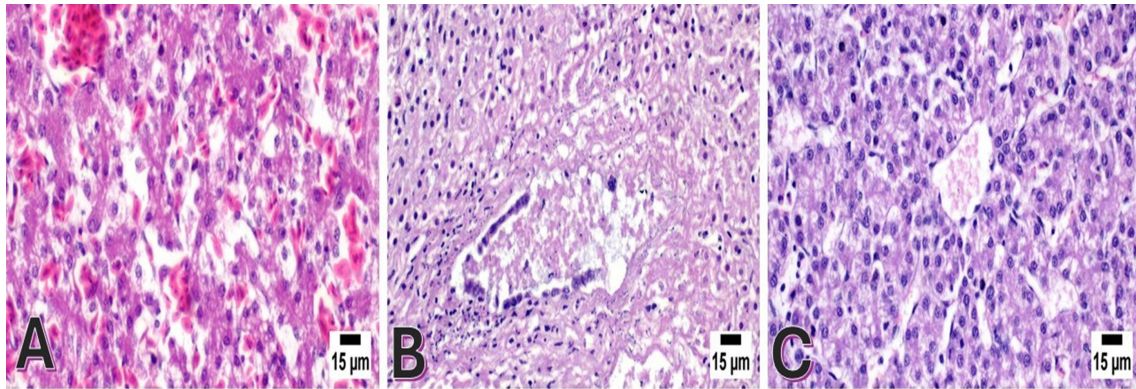


FIGURE 1

Photomicrograph of liver, (A) group (PC) at 32 days showing sinusoidal dilation associated with disarrangement of hepatic plates (H&E). (B) group (NC) at 32 days showing extensive necrosis of the periportal hepatic plates with pyknotic nuclei and abundant eosinophilic tissue debris (H&E). (C) group (NC + emulsifier) at 32 days showing normal histological structure of hepatic parenchyma (H&E).

Lysophospholipids possess amphiphilic properties that reduce surface tension and improve lipid dispersion in the intestinal lumen, thereby increasing the surface area available for enzymatic action (Guerreiro Neto et al., 2011; Zhao and Kim, 2017). In parallel, exogenous lipase directly catalyzes the hydrolysis of triglycerides into monoglycerides and free fatty acids, facilitating micelle formation and intestinal absorption (Krogdahl, 1985b; Grulkowski, 2018). Although previous studies have reported improvements in feed efficiency and body weight gain following emulsifier supplementation (Roy et al., 2010; Brautigam et al., 2017), most investigations evaluated emulsifiers or lipase independently. The present findings therefore highlight the importance of simultaneously improving both physical and enzymatic stages of lipid digestion, particularly in diets with reduced energy density.

Reducing dietary energy significantly decreased average daily gain, whereas supplementation with either lysophospholipids or lipase partially alleviated this decline. The combined inclusion of both additives produced the greatest significant improvement in growth performance. Because average daily feed intake was not significantly affected, the observed performance enhancement can

be attributed primarily to improved nutrient utilization rather than increased feed consumption. Similar responses have been reported in broilers receiving digestive enzyme supplementation in low-energy diets (Cowieson and Adeola, 2005; Ravindran et al., 2016). Conversely, some studies reported inconsistent responses when bile acids, CSL, or lipase were used individually (Arshad et al., 2020; Wickramasuriya et al., 2020), suggesting that isolated supplementation may not sufficiently overcome the physiological limitations of lipid digestion in young broilers.

Young birds typically exhibit limited endogenous lipase secretion and reduced bile salt availability during early development (Krogdahl, 1985b; Nitsan et al., 1991), which restricts lipid digestion efficiency. The combined supplementation strategy likely compensates for these limitations by enhancing both lipid emulsification and hydrolytic capacity. This mechanism is supported by the improved retention of crude protein and ether extract observed in the supplemented groups. Enhanced lipid digestion may indirectly improve protein utilization by reducing interference of undigested fat with proteolytic enzyme activity and improving intestinal absorptive capacity (Maldonado-Valderrama

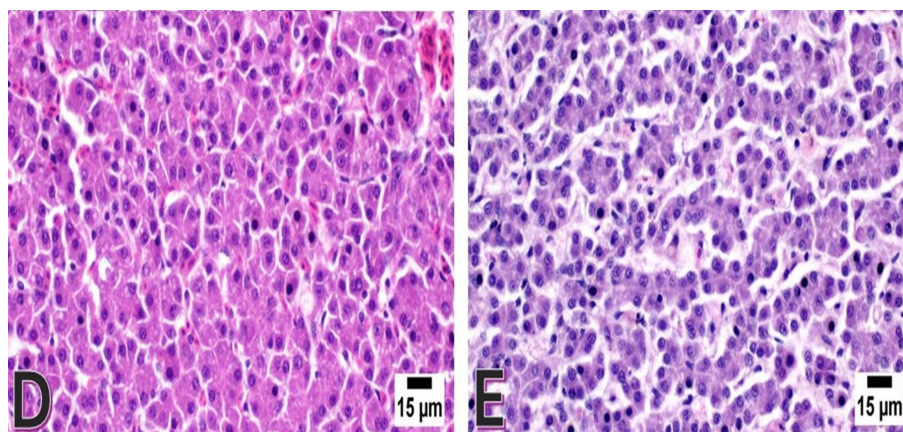


FIGURE 2

Photomicrograph of liver, (D) group (NC + lipase) at 32 days showing normal hepatocytes arranged in hepatic plates. Each hepatic plate is two cells thick, with bile canaliculus in the middle. Sinusoids lie between the hepatic plates (H&E). (E) group (NC + emulsifier+ lipase) at 32 days showing normal hepatic plates (H&E).

et al., 2011; Zaefarian et al., 2015). Similar improvements in nitrogen retention following lysolecithin supplementation have been reported in pigs and poultry (Jones et al., 1992; Papadopoulou et al., 2018). Enhanced micelle formation also facilitates absorption of long-chain fatty acids and fat-soluble nutrients (Carey and Small, 1978; Jansen et al., 2015), while the release of free fatty acids during lipase-mediated hydrolysis may further stabilize micellar structures and amplify lipid solubilization (Rodriguez-Sanchez et al., 2019).

Improved lipid digestion may also influence gastrointestinal microbial activity. Undigested dietary fat reaching the hindgut can disrupt microbial balance and promote undesirable fermentation processes (Kiarie et al., 2013; Apajalahti and Vienola, 2016). By increasing lipid digestion efficiency in the small intestine, combined supplementation likely contributes to a more stable intestinal environment. This interpretation is supported by the restoration and enhancement of villus height observed in supplemented birds. Energy restriction significantly impaired intestinal epithelial development, as rapidly proliferating enterocytes require adequate nutrient and energy supply (Uni et al., 2005). Enhanced villus architecture increases absorptive surface area and contributes directly to improved feed efficiency (Awad et al., 2009; Wang et al., 2024).

Despite improvements in growth performance, carcass yield and muscle distribution were not significantly affected by dietary treatments. This indicates that enhanced performance was primarily driven by improved nutrient utilization rather than major alterations in carcass composition. Reduced abdominal fat deposition in energy-restricted groups suggests changes in nutrient partitioning toward lean tissue accretion (Noblet et al., 1994). Improved lipid digestion may also reduce hepatic lipogenesis by enhancing peripheral fatty acid utilization (Hermier, 1997a; Hermier, 1997b), thereby contributing to lower adiposity.

The increased relative gizzard weight observed in lipase-supplemented birds may reflect adaptive responses in digestive physiology, as dietary enzymes and feed additives have been shown to influence gastrointestinal organ development (Svihus, 2011). Serum biochemical parameters further supported the beneficial metabolic effects of supplementation. Lower concentrations of total cholesterol and triglycerides in supplemented birds are consistent with improved lipid metabolism reported in previous emulsifier studies (Roy et al., 2010; Brautigam et al., 2017; Hu et al., 2018). Reduced triglyceride levels may indicate enhanced peripheral uptake and utilization rather than decreased hepatic synthesis.

Improved lipid clearance and reduced hepatic lipid accumulation may explain the observed reduction in GOT activity, suggesting alleviation of metabolic stress on hepatocytes (Sies, 1997; Attia et al., 2017). Importantly, stable HDL and LDL concentrations across treatments indicate that lipoprotein metabolism remained physiologically balanced, suggesting improved lipid turnover without disruption of normal transport mechanisms.

The enhanced antioxidant status observed in supplemented birds, reflected by increased glutathione peroxidase activity and reduced malondialdehyde concentrations, indicates lower oxidative stress and lipid peroxidation. Rapid growth and high metabolic rates increase reactive oxygen species production in broilers (Surai,

2016). Improved lipid digestion may reduce accumulation of oxidizable lipids within tissues, thereby lowering oxidative burden. Additionally, phospholipids present in lysophospholipid supplements may exert intrinsic antioxidant effects by stabilizing cellular membranes and scavenging free radicals (King et al., 1992). Reduced oxidative damage has also been associated with improved immune competence and meat quality, including extended shelf life due to decreased lipid oxidation during storage (Lin et al., 2006; Falowo et al., 2014).

At the molecular level, energy restriction significantly downregulated IGF-1 expression, indicating suppression of anabolic growth pathways. IGF-1 is a central mediator of growth hormone activity and regulates cellular proliferation, protein synthesis, and muscle development (Duclos, 2005; Yaqoob et al., 2022). Restoration and upregulation of IGF-1 expression in supplemented birds suggest that improved nutrient availability reactivates anabolic signaling pathways such as PI3K–Akt–mTOR (Vary and Lynch, 2007). These findings highlight that nutritional interventions can influence growth not only through digestive mechanisms but also via endocrine and nutrigenomic regulation (Bottje and Carstens, 2009).

Overall, the findings support a multi-level mechanism through which combined lysophospholipid and lipase supplementation enhances broiler performance under reduced-energy feeding conditions. Improved lipid emulsification and triglyceride hydrolysis enhance micelle formation and nutrient absorption (Nitsan et al., 1991; Noy and Sklan, 1995; Zhao and Kim, 2017), leading to increased protein and energy retention (Maldonado-Valderrama et al., 2011; Zaefarian et al., 2015), reduced hepatic metabolic stress (Sies, 1997), strengthened antioxidant defenses (King et al., 1992; Surai, 2016), improved intestinal morphology (Uni et al., 2005; Awad et al., 2009), and activation of anabolic growth signaling pathways (Vary and Lynch, 2007; Yaqoob et al., 2022). This coordinated physiological response explains the superior performance achieved with combined supplementation compared with individual additive strategies.

From a practical and sustainability perspective, these results are highly relevant to modern poultry production requirements. Feed represents the largest production cost, and dietary energy is among the most expensive nutrients in broiler diets (Leeson and Summers, 2005). Strategies that improve nutrient utilization while allowing reductions in dietary energy density can therefore reduce feed costs and improve production efficiency. Additionally, improved digestibility may decrease nutrient excretion into the environment, supporting more sustainable poultry production systems (Nahm, 2007; Ravindran et al., 2016).

In conclusion, the present findings demonstrate that the combined supplementation of lysophospholipids and exogenous lipase represents an effective nutritional strategy to enhance lipid utilization and growth efficiency in broilers fed reduced-energy diets. The synergistic improvement in lipid emulsification, triglyceride hydrolysis, nutrient absorption, antioxidant stability, intestinal (Attia and Kamel, 2012; Al-Daraji et al., 2010) integrity, and anabolic signaling provides a comprehensive physiological explanation for the superior performance observed. These results confirm that optimizing digestive efficiency can compensate for

reductions in dietary energy density, thereby improving feed efficiency and economic returns while supporting more sustainable poultry production systems through reduced nutrient excretion (Carey and Small, 1978; King et al., 1992; Noy and Sklan, 1995; Sies, 1997; Nahm, 2007; Vary and Lynch, 2007; Awad et al., 2009; Maldonado-Valderrama et al., 2011; Surai, 2016; Zhao and Kim, 2017; Yaqoob et al., 2022; Mohiti-Asli et al., 2025).

Conclusions and application

1. The main results of this study demonstrate that combined lysophospholipid and lipase supplementation represents an effective strategy for maintaining broiler performance under energy-restricted feeding conditions. The synergistic benefits observed with combined supplementation exceed those achieved with individual additives, supporting the development of integrated nutritional management approaches.
2. The comprehensive improvements observed across multiple parameters, including growth performance, nutrient utilization, metabolic health, intestinal morphology, and gene expression demonstrate that appropriate supplementation strategies can optimize broiler production at multiple biological levels.
3. These findings provide a scientific foundation for sustainable broiler nutrition management strategies that balance economic efficiency with animal welfare and product quality considerations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The animal study was approved by This research was conducted following the animal welfare guidelines established by Damanhour University, Egypt, with all procedures approved by the Institutional Animal Care and Use Committee (DMU/VetMed-2025/017). The study employed a completely randomized design to evaluate five dietary treatments over a 32-day experimental period. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

WF: Formal analysis, Writing – original draft, Writing – review & editing, Conceptualization. MA-L: Writing – review & editing, Methodology, Writing – original draft, Validation, Conceptualization.

MK: Methodology, Writing – original draft, Conceptualization, Resources, Validation. FS: Supervision, Writing – review & editing, Resources, Funding acquisition. LD: Writing – review & editing. AS: Conceptualization, Project administration, Methodology, Writing – review & editing. HA: Writing – review & editing, Supervision, Investigation, Data curation.

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Conflict of interest

Authors WF, FS, and LD were employed by Devenish Nutrition Ltd.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2026.1790504/full#supplementary-material>

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