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Evaluation of Low Energy Diet Supplemented with Lipase Enzyme and Emulsifier on Performance, nutrient digestibility, Antioxidant Capacity and Liver Gene Expression in Broilers

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ABSTRACT
This study aimed to investigate the effects of a low-energy (LE) diet supplemented with lipase and an emulsifier (lysolecithin) on growth performance, relative organ weight, antioxidant capacity, and liver gene expression in broilers. Three hundred and sixty-day-old broiler chicks were divided into six dietary treatments, with six replicates of ten birds each. Two-phase diets were formulated: starter (0-21 days) and finisher (22-35 days). The dietary treatments were follows as; T_1 (basal diet), T_2 (low energy,– 100 kcal/kg from BD), T_3 (LE + 0.04% lipase), T_4 (LE + 0.04% lysolecithin), T_5 (LE + 0.04% lipase and lysolecithin), T_6 (LE + 0.08% lipase and lysolecithin). The low-energy diet supplemented with 0.08% lipase & lysolecithin showed significantly ($P<0.05$) higher weight gain and feed intake than the other treatment groups. However, the dietary energy levels did not affect the feed conversion ratio. Similarly, significant ($P<0.05$) impact of low-energy diet containing 0.08% lipase and lysolecithin was noted on ether extract digestibility. However, relative organ weight of broilers was not significantly ($P>0.05$) affected among the different dietary treatment groups. Dietary treatments did not affect significantly ($P>0.05$) antioxidant capacity of broilers. Similarly, the effect of different dietary treatments on liver genes (FAS and FABP) in broilers was also non-significant ($P>0.05$). It can be concluded that a low-energy diet supplemented with 0.08% lipase and lysolecithin could exhibit better growth performance in broilers.

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I. INTRODUCTION

In modern feed formulation practices, cost savings are one of the biggest challenges for nutritionists while maintaining the performance of broilers. Different feed additives, such as Phytase, NSP enzymes, probiotics, and prebiotics, are used to minimize the feed cost. Among the various feed additives, lipase and emulsifiers are the least studied and less commonly used in commercial feeds. Broilers need high-energy-density diets to meet their energy requirements because of the improved genetics of broilers for better growth performance. Vegetable and animal fats are used as sources of energy in broilers to improve growth performance (Lesson & Summers, 2005; Abudabos, 2014). However, adding a high level of fat to the diet of broilers can affect nutrient digestibility (Trancharoenrat et al., 2013; Siyal et al., 2017), especially during the early life of broiler chickens (Ravindran et al., 2016). According to some researchers (Roy et al., 2010; Lilburn & Loeffler, 2015; Classen, 2017), poor digestion and absorption of fat in young broilers could be due to reduced secretion of pancreatic lipase and bile salts. These limitations can be overcome by supplementing broiler diets with exogenous lipase (Adeola & Cowieson, 2011) and emulsifiers (lysolecithin, lysophospholipids, or bile salts) to improve micelle formation, thereby increasing fat digestion (Jansen et al., 2015; Wang et al., 2016; Zhao & Kim, 2017).

Haetinger et al. (2021) supplemented the lysophospholipids diet and found improved growth performance, nutrient digestibility, and better economics in broilers. Similarly, Wealleans et al. (2020) studied the effect of lysolecithin supplementation in broiler diets and reported improved energy and nutrient utilization. Similarly, the addition of soy lecithin alone or in combination with lipase to broiler diets improved growth performance and antioxidant capacity (Nagargoje et al., 2016). Similarly, other researchers (Melegy et al., 2010; Zhang et al., 2011; Jansen et al., 2015) have also reported a positive effect of emulsifier supplementation in broilers. Therefore, the addition of lipase and emulsifiers to broiler diets can overcome the digestion and absorption limitations of fats. According to Roy et al. (2010), supplementing broiler diets with exogenous emulsifiers has beneficial effects on live weight, yield, and abdominal fat. However, Huang et al. (2008) did not observe a significant effect of supplementing broiler diets with soy lecithin on abdominal fat and liver fat. Similarly, other researchers found that the relative organ weight was not affected by the addition of an emulsifier to the diet of broilers (Roy et al., 2010; Guerreiro-Neto et al., 2011; Cho et al., 2012; Abbas et al., 2016). According to Huang et al. (2008), the expression of liver genes FAS and FABP could be affected by feeding broiler chickens a lysolecithin-containing diet. On the other hand, Ge et al. (2019) reported that the gene expression of FAS and

FABP was reduced by supplementing broiler diets with emulsifiers. Similarly, Piekarski et al. (2016) observed that chenodeoxycholic acid decreases ACC and FAS gene expression in broilers. In contrast, Kubis et al. (2022) reported no significant difference in gene expression in broilers at the age of 35 days.

As there are limited available data regarding emulsifier and/or lipase supplementation in broiler diets, the objective of the present study was to evaluate the effect of emulsifier and/or lipase supplementation on performance, antioxidant capacity, relative organ weight, and liver gene expression in broiler chickens.

II. MATERIALS AND METHODS

The study was conducted at the Experimental Broiler House, Department of Animal Nutrition, SAU, Tandojam. In this experiment, the birds had free access to feed and water. The light was provided to the birds round the clock along with optimized environmental and hygienic conditions. The vaccination schedule was applied according to the recommendations of the Pakistan Poultry Association (PPA) for that area. Two-phase diets were formulated i.e. starter (0-21 days) and finisher (22-35 days). The six dietary treatments were: i) basal diet (ME= 2900 and 3100 kcal/kg for starter and finisher diet, respectively), ii) low energy diet (LE; ME= 2800 and 3000 kcal/kg for starter and finisher diet, respectively), iii) LE diet supplemented with 0.04% lipase (LEL), iv) LE diet containing 0.04% lysolecithin (LEL), v) LE diet containing both 0.04 % lipase and lysolecithin vi) LE diet supplemented with 0.08% lipase and lysolecithin.

Table 1- Ingredient and nutrient composition of experimental diets for broiler chicks (as-fed basis)

Items	**Phase 1 (d 1 to d 21)**		**Phase 2 (d 22 to d 35)**	
	CON [1]	**LE [2]**	**CON**	**LE**
Ingredients%				
Maize	45.55	42.95	57.74	54.99
Rice polishing	7.74	10.00	1.00	5.00
Soybean meal	38.7	39.24	32.84	32.66
Soy oil	3.20	3.00	4.10	3.00
Limestone	1.64	1.64	1.41	1.43
Di-calcium phosphate	1.90	1.90	1.86	1.86
Sodium chloride	0.37	0.38	0.37	0.37
Sodium bicarbonate	0.06	0.06	0.06	0.06
L- Lysine sulphate	0.43	0.42	0.28	0.29
DL-Methionine	0.31	0.31	0.24	0.24
Vit. & Min. Premix[3]	0.10	0.10	0.10	0.10
Calculate nutrient composition (%)				
ME (Kcal/Kg)	2900	2800	3100	3000
Crude Protein	22	22	20	20
Ether extract	6.25	6.04	6.67	5.97
Calcium	0.99	0.99	0.92	0.92
Available Phosphorous	0.45	0.45	0.5	0.5
Analyzed nutrient composition (%)				
Dry matter	89.52	89.76	89.91	90.63
Crude Protein	22.35	22.15	20.25	20.10
Ether Extract	6.89	6.24	6.54	6.22
Ash	4.89	525	4.51	4.86

[1] Basal diet, [2] Low energy, [3] Nutrient level of premix (per kg diet): vitamin A , 15000 IU; vitamin D3, 5,000 IU; vitamin E, 40 mg; vitamin K, 3 mg; vitamin B1, 3 mg; vitamin B2, 12 mg; vitamin B3, 60 mg; vitamin B5, 15 mg; vitamin B6,4 mg; Biotin, 0.2 mg; Folic acid, 3mg; vitamin B12, 0.03 mg, choline 1850 mg, Iron, 25 mg; copper, 15 mg; zinc, 100 mg; manganese, 110 mg; iodine, 1 mg; selenium. 0.4 mg

DATA COLLECTION

The performance of the birds was recorded weekly by observing their body weights. FCR was also determined at the end of each week. The dry matter, crude protein, ether extract, and ash content of the feed were analyzed according to the methods of the Association of Official Analytical Chemists (AOAC, 2005).

When birds reached at 32th day of age, three birds/replicate were randomly selected. For total fecal collection, these birds were assigned metabolic cages. One day as an adaption periods was given to the birds. From days 33rd-35th, total fecal collection was done and then collected samples were kept in hot air oven for a period of 72 hours at 65 ˚C for drying. After drying, samples were analyzed. To determine digestibility a method described by Perez et al. (1995) was applied.

Nutrient digestibility (%) = (Nutrient intake – nutrient excreted/Nutrient intake) × 100

On day 35 of the feeding trial, chickens were prohibited from feeding for six hours with free access to water. Afterward, two birds per replicate were randomly selected, weighed, and slaughtered by serving of jugular vein. After slaughtering, birds were de-feathered, and dressing percentage, abdominal fat, breast, thigh, drumstick, heart, liver (without gallbladder), gizzard (removal of content), intestinal weight (removal of the content), and intestinal length (cm) were recorded. Relative weights of organs were determined using a weighing balance with an accuracy of 0.1 g as a gram of the organ/kg of body weight.

The enzyme activity of antioxidants such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GSH-PX) in the serum was evaluated spectrophotometrically using commercial kits according to the manufacturer's protocol.

When the birds reached the 35th day of age, tissue samples of the liver were collected from one bird per replicate. After being sub-packed in aliquots and snap-frozen in liquid nitrogen, all samples were stored at -70°C until further analysis. RT-PCR was used to detect mRNA expression at the transcriptional level in the liver of broilers. Total RNA was isolated from the liver of birds using TRIzol reagent (Takara, Tokyo, Japan) according to the manufacturer's instructions. Total RNA was treated with 100 U DNase I (RNase Free; Takara, Tokyo, Japan) for 30 min at 37°C to ensure that all total RNA was free of genomic DNA contamination. The total RNA concentration was quantified using a NanoDrop spectrophotometer (ND-1000 Spectrophotometer, Rockland, DE, USA). The absorption ratios (260/280 nm) of all preparations were between 1.8 and 2.0. Each RNA sample was subjected to electrophoresis on 1.4% agarose formaldehyde gel to verify its integrity. Single-stranded cDNAs was synthesized and real-time PCR was performed. The negative controls involved the omission of RNA from the RT reactions and amplification with specific primer/probe sets to confirm the lack of genomic DNA contamination. Primers specific for nutrient transporter genes (FABP: Fatty Acid Binding Protein; FAS: Fatty Acid Synthase) were designed as described previously by Gilbert et al. (2008) and commercially synthesized for RT-PCR. The PCR products were sequenced to validate amplicon identity. The 2ΔΔCt method (Livak & Schmittgen, 2001) was used to analyze the RT-PCR data.

Statistical Analysis

The collected data were analyzed by analysis of variance (ANOVA) using SAS 9.1. Significant means were compared using Duncan's Multiple Range (DMR) (Duncan, 1955) and LSD.

III. Results

Growth performance

In the overall experimental period, the low-energy diet supplemented with 0.08% lipase & lysolecithin showed significantly ($P<0.05$) higher weight gain and feed intake than the other treatment groups. Significantly reduced weight gain and feed intake were observed in broilers fed the control diet containing high dietary energy. However, the effects of the experimental diets on the FCR were not significant ($P>0.05$).

Table 2- Effects of low-energy diet supplemented with lipase and lysolecithin on growth performance of broiler chickens

Items	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-Value
0-21 days								
DFI	51.32	56.9	57.08	58.16	56.37	58.81	0.87	0.2
DWG	42.98	45.45	47.39	46.97	45.05	48.3	0.61	0.85
FCR	1.19	1.25	1.21	1.24	1.25	1.22	0.01	0.08
22-35 days								

DFI	146.99	152.11	159.23	156.38	157.43	161.42	1.57	0.7
DWG	90.92	93	90.79	92.76	91.27	95.41	0.64	0.99
FCR	1.62	1.64	1.75	1.69	1.73	1.69	0.02	0.71
0-35 days								
DFI	89.59^{b}	97.98^{a}	97.95^{a}	97.45^{a}	96.80^{a}	99.86^{a}	0.85	0.01
DWG	62.16^{b}	66.97^{a}	64.75^{ab}	66.28^{a}	65.79^{a}	67.15^{a}	0.5	0.02
FCR	1.44	1.47	1.51	1.47	1.47	1.49	0.01	0.88

T_1= Basal diet, T_2= low energy, T_3= low energy + 0.04% lipase, T_4= low energy + 0.04% lysolecithin, T_5= low energy + 0.02% lipase and 0.02% lysolecithin, T_6= low energy + 0.04% lipase + 0.04% lysolecithin. ab Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of mean.

Nutrient digestibility

The influence of different dietary treatments on nutrient digestibility (dry matter, crude protein & crude fat) in broiler chickens is summarized in Table 3. Higher but statically non-significant ($P>0.05$) dry matter and crude protein digestibility was recorded in the group of birds fed a diet containing low energy supplemented with 0.08% lipase & lysolecithin . However, the effect of different treatments on crude fat digestibility was significant ($P<0.05$). Highest crude fat digestibility was noted in group of broilers fed 0.08% lipase and lysolecithin supplemented diet.

Table 3- Effect of different oil sources on nutrient digestibility of broilers

Items (%)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**SEM**	***P*-value**
Dry matter	78.92	79.26	80.00	81.04	80.94	82.96	0.61	0.06
Crude protein	78.75	78.80	80.19	79.41	79.17	81.46	0.44	0.16
Crude Fat	76.35^{b}	78.81^{ab}	79.89^{ab}	79.27^{ab}	80.28^{ab}	83.06^{a}	0.81	0.039

T_1= Basal diet, T_2= low energy, T_3= low energy + 0.04% lipase, T_4= low energy + 0.04% lysolecithin, T_5= low energy + 0.02% lipase and 0.02% lysolecithin, T_6= low energy + 0.04% lipase + 0.04% lysolecithin. ab Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of mean.

Relative organ weight

The relative weights of the different organs (breast, thigh, drumstick, abdominal fat, heart, liver, spleen, thymus, bursa, intestinal weight, & intestinal length) were not affected by the experimental diets.

Table 4- Effects of low-energy diet supplemented with lipase and lysolecithin on relative organ weight in broiler chickens

Items (%)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**SEM**	***p*-Value**
Breast (without bone)	28.15	26.69	28.41	28.63	29.12	29.74	0.33	0.91
Thigh	7.92	7.22	7.62	7.21	6.92	7.59	0.12	0.18
Drumstick	4.50	4.10	4.76	4.62	4.35	4.66	0.16	0.86
Abdominal fat	1.78	1.61	1.26	1.19	1.21	0.93	0.05	0.26
Heart	0.48	0.55	0.49	0.61	0.58	0.49	0.02	0.11
Liver	2.35	2.56	2.45	2.68	2.47	2.61	0.06	0.8
Spleen	0.1	0.09	0.1	0.1	0.1	0.09	0.04	0.94
Gizzard	1.31	1.38	1.35	1.27	1.43	1.42	0.03	0.82
Bursa	0.14	0.13	0.15	0.18	0.18	0.17	0.06	0.57
Thymus	0.2	0.21	0.2	0.21	0.21	0.2	0.04	0.66
Intestinal weight	5.41	6.14	6.44	5.63	5.81	6.08	0.13	0.23
Intestinal length (cm)	157	166	162	182	172	179	4.95	0.6

T_1= Basal diet, T_2= low energy, T_3= low energy + 0.04% lipase, T_4= low energy + 0.04% lysolecithin, T_5= low energy + 0.02% lipase and 0.02% lysolecithin, T_6= low energy + 0.04% lipase + 0.04% lysolecithin. ab Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of mean.

Antioxidant capacity

The effects of the dietary treatments on the antioxidant capacity of broiler chickens are shown in Table 3. The effects of the low-energy diet and supplementation with lipase & lysolecithin were non-significant ($P>0.05$) on superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and GSH-PX.

Table 5- Effects of low-energy diet supplemented with lipase and lysolecithin on anti-oxidant capacity in broiler chickens

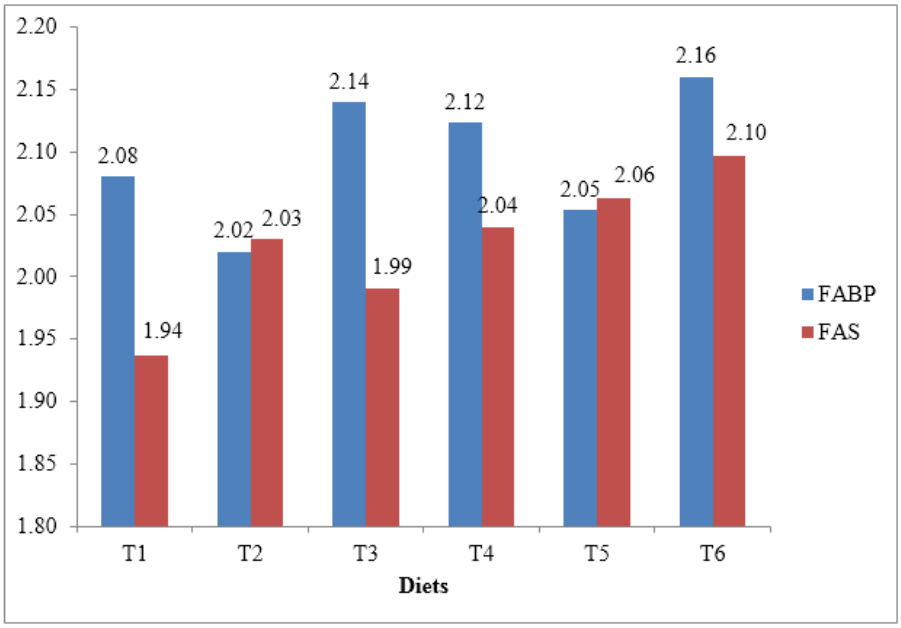
Items	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-Value
SOD (U/ml)	324.67	326.33	355.33	377.67	415.67	353.33	31.28	0.98
CAT (U/ml)	2	2.21	2.12	2.42	2.17	2.27	0.07	0.62
MDA (nmol/ml)	4.23	4.18	3.78	3.83	3.74	3.13	0.15	0.89
GSH-PX (U/ml)	332.67	390	374.67	354.33	388.67	335.67	9.9	0.28

T_1= Basal diet, T_2= low energy, T_3= low energy + 0.04% lipase, T_4= low energy + 0.04% lysolecithin, T_5= low energy + 0.02% lipase and 0.02% lysolecithin, T_6= low energy + 0.04% lipase + 0.04% lysolecithin. ab Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of mean.

Liver gene expression

In our study, birds fed a low-energy diet supplemented with lipase & lysolecithin did not show significant ($P>0.05$) effects on liver gene expression. However, fatty acid-binding protein (FABP) and fatty acid synthase (FAS) gene expression was higher in birds fed a low-energy diet containing 0.08% lipase and lysolecithin.

Figure 1- Effects of low-energy diet supplemented with lipase and lysolecithin on liver gene expression in broiler chickens



T_1= Basal diet, T_2= low energy, T_3= low energy + 0.04% lipase, T_4= low energy + 0.04% lysolecithin, T_5= low energy + 0.02% lipase and 0.02% lysolecithin, T_6= low energy + 0.04% lipase + 0.04% lysolecithin. ab Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of mean.

IV. DISCUSSION

Growth performance

In the present study, feed intake & weight gain were improved by 0.08% lysolecithin and lipase supplementation in a low-energy diet in broilers. However, higher feed intake and weight gain were recorded in broilers fed a low-energy diet with or without supplementation of exogenous lipase and lysolecithin compared to the control group. The reduced feed intake and body weight gain in the control diet could be due to the high-energy diet satisfying the energy requirements of birds. The findings of our study are similar to those of Lamot et al. (2017), who reported that increasing dietary energy can reduce feed intake in broilers. Kubis et al. (2022) observed that dietary supplementation of emulsifiers significantly ($P<0.05$) influenced body weight gain and overall FCR in broilers. Contradicting our findings, some researchers have reported that feeding low-energy diets reduced body weight gain compared to high-energy diets during the 14 days of the experimental period (Zhao & Kim, 2017; Hu et al., 2018) and–28-35 days (Ge et al., 2019). In the present study, supplementation with 0.08% lipase and emulsifier in a low-energy diet significantly improved weight gain and feed intake in broilers. Similar results have been reported by Haetinger et al. (2021), who reported that lysophospholipid supplementation can improve the growth rate, particularly when supplemented "on top" in broilers (Zaefarian et al., 2015; Zampiga et al., 2016). Similarly, several scientists have reported positive results by adding emulsifiers to broiler diets with reduced oil contact (Zhao & Kim, 2017; Chen et al., 2019).

Contrary to our findings, Vieira et al. (2012) reported that feed consumption did not change when birds were fed an emulsifier for up to 35 days of age. In an experiment, Wickramasuriya et al. (2020) fed a diet containing lipase and lysolecithin either combined or alone did not affect growth rate and FCR in broilers, regardless of dietary energy level. However, Kaczmarek et al. (2015) reported that emulsifier addition does not affect feed intake in broilers. Similarly, Kamran et al. (2020) did not observe an improvement in growth performance in broilers fed both lipase and emulsifier in a low-energy diet. Similarly, sodium stearoyl-2 lactylate, added as an emulsifier, did not improve the growth performance of broilers (Cho et al., 2012). Gurreiro et al. (2011) stated that some emulsifiers only benefit birds when soy oil is also supplemented in the diet of broilers. Similarly, Zhang et al. (2011) reported that the function of lysophosphatidyl choline as an emulsifier depends on the type of oil used in the broiler feed. In a study, Ahmad et al. (2023) reported that birds fed with lipase and emulsifier in low-energy diet exhibited higher weight gain and feed intake. In our study, better growth performance by supplementing 0.08% lipase and lysolecithin in low-energy diet could be due to increased nutrient utilization in birds.

Impact of treatment diets on nutrient utilization (DM, CP & EE) is presented in Table 3. Feeding emulsifier & lipase at 0.08% showed higher dry matter and crude protein digestibility in broilers. There was a significant ($p<0.05$) impact of treatments on digestibility of ether extract digestibility in broilers by adding 0.08% lipase and lysolecithin in low-energy diet.

Findings to our study are similar to those reported that during the age of 21 days, supplementation of 250g/ton lysolecithin exhibited better digestibility of dry matter, crude fat and AME utilization (Wealleans et al., 2020). Low-energy density diet containing 250g/ton lysolecithin could mitigate the effects of low-energy and maintains the growth performance in broilers (Papadopoulos, 2018). On a contrary, non-significant effects of supplemental lysolecithin have been reported by Khonyoung et al. (2015). Lysolecithin supplementation not only improves digestion of fats but also increases the villus length due to increased digestibility and utilization of energy (Brautigan et al., 2017). Therefore, lecithin could be incorporated to the diet to get better fat utilization. Al-Marzooqi & Leeson (2000) reported improved nutrient utilization & increased liver weight by increasing lipase concentration with 4% animal-vegetable blend of supplemented fat. Digestibility of polyunsaturated fatty acids (PUFA) was enhanced by adding emulsifier (Kubis et al., 2022).

Similar to our findings, other researcher, Brautigan et al. (2017) & Bontempo et al. (2018) indicated better nutrient take up from gut by adding emulsifier in diet. Similarly, Wealleans et al. (2020) also reported increased crude fat and AME digestibility due to emulsifier. In a low fat containing diet, addition of emulsifier improved nutrient digestibility (Adrizal et al., 2002). Likewise, improved nitrogen retention was noted by feeding low-energy diet with emulsifier (Gheisar et al., 2015). Our findings are consistent to those who reported improved nutrient retention by incorporating emulsifier (Siyal et al., 2017). On a contrary, Hu et al. (2018) observed no impact on nutrition digestibility through supplementation of lipase with low-energy diet in broiler chickens. Similarly, lipase addition had no impact on nutrient retention in young broilers when exogenous lipase was added (Meng et al., 2004). A low-energy diet showed improved nutrient retention in broilers when emulsifier was supplemented (Wickramasuriya et al., 2020).

A low-energy diet supplemented with lysolecithin & lipase did not affect the relative organ weight in broiler chickens in our study. Similar findings were reported by Ge et al. (2019) and Upadhaya et al. (2019), who found that low dietary energy did not affect the liver, spleen, gizzard, abdominal fat, or bursa breast muscle weight in broilers. In the present study, the level of abdominal fat was similar between the high- and low-energy diets

due to decreased feed intake in the high-energy diet. However, some researchers have argued that an increase in dietary intake increases the proportion of abdominal fat in broiler chickens (Zhao & Kim, 2017).

The weight of the remaining organ index was not affected by the dietary intake of lysolecithin & lipase in this study, which is similar to the results of previous studies by Hu et al. (2018) and Ge et al. (2019). In contrast, Jansen et al. (2015) found a reduction in abdominal fat in broilers fed lysophospholipids 500 g/ton. On the other hand, Azman & Ciftci (2004) suggested that the addition of lysolecithin to the diet has a positive effect on carcass composition and dressing percentage in broilers.

Dietary emulsifier supplementation does not affect abdominal fat deposition in broilers (Guerreiro-Neto et al., 2011; Cho et al., 2012; Oliveira et al., 2019). However, Roy et al. (2010) reported the beneficial effects of exogenous emulsifiers on live weight gain and abdominal fat in broilers. Huang et al. (2008) observed that the proportion of abdominal fat and liver fat was not affected by adding lysolecithin to the broiler's diet. Similarly, previous studies reported that emulsifier supplementation in the diet of broilers did not affect relative organ weight in birds (Gurreiro-Neto et al., 2011; Cho et al., 2012; Abbas et al., 2016).

The effects of a low-energy diet with lipase and lysolecithin supplementation on superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and GSH-PX were non-significant ($P > 0.05$). Oxidative stress is detrimental to animals because it can decrease immune responses. However, feed additives containing antioxidants and emulsifiers are an effective strategy for reducing the effects of oxidants on broiler performance (Siyal et al., 2017; Saleh et al., 2020).

In our study, birds fed a low-energy diet supplemented with lysolecithin and lipase did not show significant ($P>0.05$) effects on liver gene expression. Our findings are similar to those reported by Kubis et al. (2022), who observed that dietary supplementation affects gene expression in broilers at the age of 35 days. Ge et al. (2019) reported that emulsifiers in broiler diets reduced the expression of FABP and FAS genes. Similarly, Piekarski et al. (2016) observed that chenodeoxycholic acid decreased ACC and FAS gene expression in broilers. In contrast, Siyal et al. (2017) concluded that emulsifier supplementation in broiler diets can positively affect liver gene expression.

V. CONCLUSIONS

It could be concluded that dietary supplementation with 0.08% emulsifier and lipase in the diet of broilers with reduced energy could exhibit better effects on the growth performance and nutrient utilization in broiler chickens. However, emulsifier and lipase supplementation in low-energy diet had no impact on relative organ weight, antioxidant activity, and liver gene expression in broilers.

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