Evaluation of fatty acid esters and emulsifier supplementation as nutritional strategies for enhancing Broiler performance

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Abstract

The present study was conducted to investigate the effect of dietary supplementation with fatty acid esters and an emulsifier on broiler performance. A total of 192 day-old broiler chicks of either sex (50% male and 50% female, in accordance with hatchery guidelines) were randomly assigned to 8 experimental groups, with 6 replicates of 4 chicks each. The standard broiler diets Control (CON) were formulated according to commercial chick feed specifications. The Antibiotic Growth Promoter (AGP) diet was identical to CON, except with the addition of an antibiotic. In contrast, MG0.8, MGR0.8, and MGH0.8 replaced the antibiotic with fatty acid esters and an emulsifier at 0.8 g/kg feed, with energy reduced by 2.5% (MGR) and 3.0% (MGH), respectively. Meanwhile, MG1.0, MGR1.0, and MGH1.0 replaced antibiotics with fatty acid esters and an emulsifier at 1.0 g/kg feed, with energy reductions of 2.5% and 3%, respectively. Individual body weight and pen feed intake of broilers were recorded, allowing for the calculation of the feed-to-gain ratio and the European Production Efficiency Index (EPEI). The findings revealed that birds fed a diet with MGH0.8 showed improved ($p<0.05$) overall growth performance economically, as indicated by body weight, weight gain, feed-to-gain ratio, EPEI, and digestibility of protein and crude fat. Carcass yield, organ weight, processing losses, and nutrient composition of meat were unaffected by the supplementation. However, significantly lower cecal, *C. perfringens* and *E. coli* counts, along with higher serum IgG and IL-10 levels, were observed in the MG1.0 supplemented diet. Broilers fed the MGH0.8 diet exhibited better feed cost per kg weight gain, a humoral immune response against Newcastle disease virus, and improved intestinal morphology (reduced crypt depth (CD), increased villus height (VH), intestinal wall thickness, and VH:CD ratio. Thus, a diet supplemented with MGH0.8 g can serve as an economically efficient alternative to antibiotic growth promoters in broilers.

Key words: Broiler, Fatty acid esters, Immunity, Intestinal histo-morphometry, Performance.

Introduction

The poultry industry plays a crucial role in the global animal husbandry sector, primarily driven by the rising demand for meat and eggs. The use of antimicrobials in poultry was banned by the EU in 2006 due to concerns over bacterial resistance and side effects (Abreu et al., 2023, Prem Kumar et al., 2026). For these reasons, alternatives to AGPs are being explored (Tsugkieva et al. 2021; Gobezie 2022). Enzymatic hydrolysis of triglycerides and diglycerides produces fatty acid esters, which exhibit amphiphilic properties. These amphiphilic characteristics of fatty acid esters act as antioxidant, antiviral, antifungal, and antibacterial agents (Rarokar et al., 2017). Fatty acid esters possess membrane-lytic properties due to their amphipathic nature, leading to instability in bacterial membranes and the formation of pores. This destabilizing effect increases cell permeability, ultimately resulting in cell lysis and death (Yoon et al., 2018). In broilers, mono-glycerol compounds reduce the levels of *C. perfringens* and *E. coli* (Skrivanova et al., 2014). According to Galli et al. (2021), incorporating fatty acid esters into the diet of broiler chickens improves their productivity and positively affects the fatty acid composition of the meat. It is considered a highly promising feed additive for chickens due to its ability to enhance productivity, support health, optimize feed efficiency, and improve meat quality (Fortuoso et al., 2019; Mustafa, 2019). Glycerol monolaurate (GML), an ester of medium-chain fatty acids, naturally occurs in coconut oil, palmetto, and human milk (Schlievert et al., 2019). It can be produced by combining lauric acid (C12) with glycerol and exhibits antimicrobial properties (e.g., against bacteria, viruses, and fungi), which has led to its widespread use in the feed industry (Mueller and Schlievert, 2015; Seleem et al., 2016). GML in broiler diets enhances intestinal health by either inhibiting the growth of harmful bacteria, such as *E. coli* (Fortuoso et al., 2019), or by promoting the proliferation of beneficial bacteria (Lan et al., 2021). The absorption of dietary fat in young birds can be improved through the supplementation of emulsifiers. Emulsifiers increase the active surface area of lipase to break down large fat globules into smaller droplets, thereby facilitating the absorption of dietary lipids (Oketch et al., 2023). Consequently, this increases the availability of metabolic energy and enhances growth performance (Tan et al., 2016).

Materials and Methods

Housing

The experimental chicks were raised in the battery brooder house. The battery brooders were cleaned, washed, and disinfected using blow lamping, and the entire house was fumigated with formaldehyde and potassium permanganate four days before the experiment began. Artificial heat (Temp. 95°F) was supplied to the chicks during the initial growth period (7 days) using electric bulbs. For the remainder of the experiment, the birds were kept at a normal temperature ranging from 83°F to 86°F.

Experimental diet

In this study, 192 broiler chicks (day old) were obtained from Phoenix Hatchery, Jabalpur (M.P.) (50% male and 50% female as per hatchery guidelines). These chicks were reared in a battery brooder facility within the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. The experimental design was completely randomized. The feed ingredients used in the experiment included corn, soybean meal, and soya oil (Table 1). The proximate composition of these feed ingredients is presented in Table 2. All day-old broiler chicks were individually weighed at the start of the experiment, and 192 birds of nearly identical weight (Range ±2g) were selected. Overall, there were eight dietary treatments. Each treatment consisted of six replicates with four chicks each. The eight isonitrogenous dietary treatments were: **CON** Control (Basal diet), in accordance with commercial chick feed specifications; **AGP**-Positive control (Basal diet + antibiotic); **MG0.8**-Basal diet + fatty acid esters with emulsifier at the rate 0.8 g/kg feed; **MGR0.8**-Basal diet + fatty acid esters with emulsifier at the rate 0.8 g/kg feed with reduced 2.5% ME; **MGH0.8**-Basal diet + fatty acid esters with emulsifier at the rate 0.8 g/kg feed with reduced 3% ME; **MG1**-Basal diet + fatty acid esters with emulsifier at the rate 1.0 g/kg feed; **MGR1.0**-Basal diet + fatty acid esters with emulsifier at the rate 1.0 g/kg feed with reduced 2.5% ME; **MGH1.0**-Basal diet + fatty acid esters with emulsifier at the rate 1.0 g/kg feed with reduced 3% ME.

Performance of Broilers

Body weight and weight gain The birds were weighed individually on a weekly basis to record the body weight of broilers until six weeks of age. Weight gain in different groups of broilers was calculated weekly using the following formula:

Body weight gain (g) = Final body weight – Initial body weight

Table 1: Ingredient composition of broiler experimental diets

Feed Ingredients	Experimental Diets (%)								
	Pre starter (0 - 14 days)			Starter (15 - 28 days)			Finisher (29 - 42 days)		
	CON	MGR (-2.5% ME)	MGH (-3.0% ME)	CON	MGR (-2.5% ME)	MGH (-3.0% ME)	CON	MGR (-2.5% ME)	MGH (-3.0% ME)
Maize	52.22	53.32	54.92	53.73	55.43	56.83	57.57	58.17	57.97
Soybean meal	41.13	41.03	40.43	37.50	37.10	36.70	33.12	32.92	33.32
Calcite/LSP	0.63	0.63	0.63	0.66	0.66	0.66	0.70	0.70	0.70
DCP	1.65	1.65	1.65	1.64	1.64	1.64	1.63	1.63	1.63
Vegetable oil	2.98	1.67	1.01	5.10	3.63	2.96	5.95	5.35	5.16
Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.21
Lysine	0.28	0.28	0.28	0.28	0.28	0.28	0.22	0.22	0.22
Soda Bicarb	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.24	0.24	0.24	0.22	0.22	0.22	0.20	0.20	0.20
Trace Mineral Premix[1]	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix[2]	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Threonine	0.07	0.07	0.07	0.05	0.05	0.05	0.02	0.02	0.02
Maduramicin	-	-	-	-	-	-	0.05	-	-
Robimidine	0.033	-	-	0.033	-	-	-	-	-
Liver tonic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Emulsifier *	0.05	-	-	0.05	-	-	0.05	-	-
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Cl_2 60 %	0.10	0.10	0.10	0.12	0.12	0.12	0.15	0.15	0.15
Coccidiostat	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00
TOTAL	100	100	100	100	100	100	100	100	100
	Nutrient composition calculated (%)								
CP (%)	22.5	22.5	22.5	21.0	21.0	21.0	19.5	19.5	19.5
ME (Mcal/kg)	3.00	2.925	2.910	3.125	3.047	3.031	3.25	3.169	3.152

* Only present in T0 and T1 diet

1 Trace mineral provided per kg diet: Manganese, 120mg; Zinc, 80mg; Iron, 25mg; Copper, 10mg; Iodine, 1mg and Selenium, 0.1mg.

2 Vitamin premix provided per kg diet: Vitamin A, 20000IU; Vitamin D3, 3000IU; Vitamin E, 10mg; Vitamin K, 2mg; Riboflavin, 25mg; Vitamin B1,1mg; Vitamin B6, 2mg; Vitamin B12,40mcg and Niacin, 15 mg.

Table 2: Nutrient composition of experimental broiler diets

Feed Ingredients	**Experimental Diets (%)**		
	Pre starter (0 - 14 days)	**Starter (15 - 28 days)**	**Finisher (29 - 42 days)**
Crude Protein	22.50	21.00	19.50
ME (Mcal/kg)	3.00	3.13	3.25
Total Lysine	1.42	1.25	1.14
Total Methionine	0.62	0.58	0.54
Ca	0.94	0.92	0.88
Available P	0.45	0.42	0.40

ME: Metabolizable Energy; Ca: Calcium; P: Phosphorus

Feed Intake Weekly feed consumption of broilers was recorded based on the feed offered and the leftover at the end of that week.

Feed to Gain Ratio (FCR) was calculated using the following formula:

$$F:G = \frac{\text{Quantity of feed consumed (g) during the week}}{\text{Gain in body weight (g) during the week}}$$

European Production Efficiency Index (EPEI) The EPEI was calculated using the formula provided by Hubbard (1999).

$$EPEI = \frac{\text{Body weight (kg)} \times \text{SR (\%)}}{\text{PP} \times \text{FCR}} \times 100$$

Where:
BW = Body weight (kg), SR = Survival rate (100% - Mortality),
PP = Production period (days), FCR = Feed conversion ratio (kg feed / kg gain)

Nutrient Utilization

To understand the utilization of nutrients (DM, CP, and EE) from various diets, a digestibility evaluation was conducted in the 6th week of the experiment. During the collection period, the amount of feed offered and leftovers were measured to determine the total feed intake per bird. Total excreta was collected and weighed, and then 100 g of thoroughly mixed excreta was taken and dried in a hot air oven for DM from each cage every 24-hour period for 3 days. Samples of feed and faeces collected from each treatment were dried in a hot air oven, finely ground per replicate, and stored for further proximate analysis in accordance with AOAC (2012).

Nutrient Composition of Meat

On day 35, birds were ethically euthanized using the electrical stunning method (50V, 15 sec.) to evaluate carcass characteristics. Additionally, a portion (10g) of breast muscle was collected and analyzed for its proximate composition (dry matter, crude protein, and crude fat) using AOAC (2012).

Carcass traits

Birds from each treatment were slaughtered on the 35th day of the experiment. Broilers were withheld from feed for twelve hours prior to slaughter. During this time, they were given clean and fresh drinking water ad-libitum. Before slaughter, each broiler was weighed, and then the birds were ethically euthanized using the electrical stunning method (50V, 15 sec.). Following this, a severe cut was made to the jugular vein, allowing for complete bleeding. After the bleeding was finished, weights were recorded. The weight was again noted after manual defeathering using hot water (50-55°C). The dressed weight was then recorded as follows:

Dressed weight = Live weight – Weight loss as blood, head, feathers, shank and wing tips
Eviscerated weight = Dressed weight – Weight of viscera
Drawn weight = Eviscerated weight + Weight of giblet
The organs (liver, heart, gizzard, giblet, and pancreas) collected at the time of slaughter had their weights recorded.

Immunological response

Humoral immune response

Broilers were vaccinated at 7 days of age with the F1 strain for ranikhet disease via the eye and at 21 days of age with the Lasota strain of Newcastle disease through drinking water. Blood samples were collected separately from the wing veins of vaccinated birds in non-heparinized test tubes on days 30 and 42 of the experiment. The serum was separated by centrifugation at 3000 rpm for 15 minutes and stored in a deep freezer at -20°C for subsequent analysis. Humoral immunity against Newcastle disease was assessed on days 30 and 42 using the haemagglutination inhibition test according to the method of the Office International des Epizooties (2004). All titres were recorded as log 10 of the reciprocal of the end-point dilution.

Weight of lymphoid organs

The weights of the bursa of Fabricius, spleen, and thymus were measured at the end of the experiment from sacrificed birds to calculate the relative lymphoid organ weights. Serum IgG and IL-10: The concentrations of immunoglobulins (IgG) and interleukin-10 (IL-10) were determined in the serum of birds using chicken-specific ELISA kits (Chongqing Biospes Co., Ltd, China).

Serum Biochemical Parameters

SGOT/AST and SGPT/ALT were measured using 2, 4-DNPH (Reitman and Frankel, 1957) with a commercial kit (Erba Diagnostics Ltd., India).

Histo-morphometrical Analysis of Intestine

The entire small intestinal tract was sampled for histo-morphometrical examination. Two-centimeter tissue samples were taken from the duodenum, jejunum, and ileum. The tissue samples were briefly preserved in 10% neutral buffered formaldehyde (NBF) for 72 hours, then processed for dehydration, clearing, and embedding in wax. Histological study was performed on 5-µm thick transverse sections (cut by a microtome), fixed on slides, and stained with hematoxylin and eosin (Drury and Wallington, 1980). The mucosal and muscular layers of the duodenum, jejunum, and ileum were examined using a digital camera attached to a Leica DM 1000 LED microscope, with the Leica Application Suite version 4.10 used for morphometric analysis as follows: villus height was measured from the tip (with lamina propria) of the villus to the base (villus crypt junction). Crypt depth was measured from the villus crypt intersection to the distal limit of the crypt, and the thickness of the tunica muscularis was defined as the distance between the lamina muscularis mucosae internally and the tunica serosa externally. Images were used to calculate the number of goblet cells.

Caecal microbiota population count

On day 35, caecal content was collected into sterilized plastic bags from the caeca of slaughtered birds. The caecal contents were immediately stored at -20°C and processed within 24 hours for the cecal microbiota population

count. Selected agar media were used for the enumeration of target bacterial groups. One gram of cecal content was taken in a sterile test tube and diluted with 10 ml of normal saline. Using separate sterile pipettes, up to 10^{-8} dilutions were prepared by transferring 1 ml of the previous dilution to 9 ml of diluent. Dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were used for the enumeration of E. coli, and aliquots of 100 µL were added to petri dishes with MacConkey agar. Plates were incubated at 37°C for 24 hours. Dilutions of 10^{-2}, 10^{-3}, and 10^{-4} were used for the enumeration of C. perfringens. Aliquots of 100 µL were added to petri dishes with tryptose-sulfite-cycloserine (TSC) culture medium and egg yolk emulsion. In the case of C. perfringens, plate incubation was performed anaerobically at 37°C for 48 hours in gas-pack anaerobic jars (Markey et al., 2013). A colony counter was used for the enumeration of bacterial colonies, and the results were expressed as log 10 colony-forming units (cfu) per gram of cecal digesta.

Economics of production

The economics of broiler production over feed cost was calculated using the cost of feed consumed per kg weight gain for each dietary treatment. The cost of feed was determined from the inclusion level of various ingredients multiplied by their market cost.

Gross return = Selling price of the obtained live weight

Net return = Gross return – Total feed cost

$$\text{Economic efficiency (\%)} = \frac{\text{Net return}}{\text{Total feed cost}} \times 100$$

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) with a completely randomized design (CRD), following the guidelines of Snedecor and Cochran (1994). This design was employed to assess the homogeneity of variance among components in experimental treatments via SPSS software. Duncan's Multiple Range Test (1955) was utilized to compare the differences between means at a 5% probability level. Variations in the data were expressed as pooled standard error of the mean (SEM), and the significance level was set at $p<0.05$.

Ethics Statement The study received approval from the Institutional Animal Ethical Committee (IAEC) under D.No.38/IAEC/Vety/2023 dated 28.08.2023, which is affiliated with CPCSEA, Ministry of Animal Husbandry, India.

Results

Growth performance

The performance of broilers in all treatment groups is presented in Table 3. The current study revealed a clear trend of increased body weight in birds supplemented with fatty acid esters and an emulsifier. The maximum and significantly ($p<0.05$) higher live weight and weight gain were observed in the group fed a diet with MGH0.8 compared to the control. Among all the weeks, during the fifth week of the study, the birds fed MG08 showed significantly higher ($p<0.05$) feed intake. Broilers fed a basal diet supplemented with varying levels of fatty acid esters and an emulsifier exhibited significantly ($p<0.05$) improved FCR starting from the second week of the experiment. The better EPEI was noted in the group receiving the MGH0.8 supplemented diet, as compared to the CON group. This result can be attributed to the higher body weight gain, improved feed-to-gain ratio, and increased survival rate. Broilers supplemented with varying levels of fatty acid esters along with emulsifier (0.8 and 1.0 g/kg diet) did not exhibit a significant ($p>0.05$) effect on dry matter utilization. The crude protein utilization was significantly ($p<0.05$) higher in MG1.0 compared to CON, while all other treatments were statistically ($p>0.05$) similar to MG1.0. The crude fat utilization was significantly ($p>0.05$) higher in the group fed MGH0.8 g compared to the CON diet. Similarly, diets supplemented with different levels of fatty acid esters along with emulsifier (0.8, 1.0 g/kg) had no significant effect ($p>0.05$) on the dry matter, crude protein, and crude fat content of meat.

Carcass Yield

Supplementation of different levels of fatty acid esters with emulsifiers doesn't have any significant effect of carcass weights, organ weight and processing losses of broilers (Table 4).

Immunological response

Supplementation of fatty acid esters along with an emulsifier affected the antibody titre against Newcastle disease virus, which was observed on the 30th and 42nd days, with results presented in Table 04. Broilers fed a basal diet supplemented with different levels of fatty acid esters and emulsifier (0.8, 1.0 g/kg) showed no significant effect ($p>0.05$) on antibody titre (log10) against Newcastle disease virus on the 30th day. Birds fed a diet with MGH0.8 had a significantly ($p<0.05$) higher antibody titre against Newcastle disease virus on the 42nd day compared to the CON group.

The results in Table 04 indicate that a diet supplemented with different levels of fatty acid esters and emulsifier (0.8, 1.0 g/kg) did not influence ($p>0.05$) thymus and spleen weight. However, a higher weight of the Bursa

Table 3: Growth performance in broilers of different treatment groups

Treatments										
Weekly	**CON**	**AGP**	**MG 0.8**	**MGR 0.8**	**MGH 0.8**	**MG 1.0**	**MGR 1.0**	**MGH 1.0**	**SEM**	***p-value***
Performance										
Live wt. (g)	2441.58^{c}	2491.86^{c}	2682.75^{ab}	2688.89^{ab}	2768.58^{a}	2646.70^{b}	2604.92^{b}	2610.17^{b}	19.16	0.000
Wt gain (g)	2390.08^{c}	2440.45^{c}	2631.42^{ab}	2637.64^{ab}	2717.33^{a}	2595.53^{b}	2553.67^{b}	2558.92^{b}	19.17	0.050
FI (g)	4187.57	4198.21	4304.08	4246.83	4232.58	4226.88	4131.67	4129.04	22.11	0.966
FCR	1.76^{a}	1.72^{a}	1.64^{b}	1.62^{bc}	1.56^{c}	1.63^{b}	1.62^{bc}	1.62^{bc}	0.01	0.399
EPEI	324.93^{c}	337.97^{c}	383.80^{b}	390.25^{ab}	415.75^{a}	379.88^{b}	376.17^{b}	377.90^{b}	4.92	0.000
Nutrient Utilization (%)										
DM	71.27	71.49	72.14	73.71	74.26	73.49	73.62	72.66	0.33	0.217
CP	73.92^{b}	75.24^{ab}	75.68^{ab}	77.01^{a}	77.06^{a}	77.29^{a}	76.13^{ab}	75.67^{ab}	0.34	0.215
EE	80.12^{b}	80.67^{ab}	81.08^{ab}	82.31^{ab}	84.01^{a}	81.50^{ab}	80.64^{ab}	81.14^{ab}	0.32	0.370
Nutrient Composition of breast muscle (%)										
DM	23.34	24.47	24.26	24.76	24.31	24.31	23.95	24.43	0.16	0.551
CP	21.07	21.99	21.50	20.41	22.23	21.17	20.13	20.10	0.24	0.197
EE	5.67	5.91	5.97	5.83	5.93	5.58	5.91	5.21	0.08	.400
Economics of broiler production										
Economic efficiency	6.19^{c}	7.23^{c}	12.17^{b}	21.08^{a}	24.90^{a}	11.91^{b}	20.26^{a}	20.86^{a}	1.09	0.000
Feed cost Rs/kg b.wt gain	73.14^{a}	71.67^{ab}	69.20^{bc}	64.02^{d}	62.04^{d}	69.32^{bc}	67.13^{c}	66.92^{c}	0.58	0.000

Means within the same row with different superscripts are significantly different ($p<0.05$); FI: Feed Intake; FCR: Feed Conversion ratio; EPEI: European production efficiency index; DM: Dry matter; CP: Crude Protein; EE: Ether Extract

Table 4: Carcass yields (% of live weight) of broilers in different treatment groups

Treatments										
Parameters	**CON**	**AGP**	**MG0.8**	**MGR0.8**	**MGH0.8**	**MG1**	**MGR1**	**MGH1**	**SEM**	***p-value***
Carcass weights (% of live weight)										
Dressed wt	81.89	83.57	82.45	83.69	83.08	83.35	83.34	83.13	0.21	0.447
Eviscerated wt	77.46	78.99	77.89	78.92	78.67	78.92	78.43	78.68	0.20	0.605
Drawn wt	81.63	83.35	82.20	83.43	82.85	83.10	83.07	82.87	0.21	0.427
Organ weights (% of dressed weight)										
Heart	0.71	0.61	0.63	0.66	0.64	0.59	0.69	0.62	0.01	0.418
Gizzard	2.20	2.29	2.53	2.76	2.21	2.23	2.58	2.26	0.07	0.451
Liver	2.18	2.33	2.05	2.06	2.15	2.19	2.31	2.21	0.04	0.749
giblet	5.09	5.21	5.22	5.39	5.01	5.02	5.56	5.04	0.08	0.798
Pancreas	0.31	0.26	0.31	0.31	0.29	0.30	0.32	0.31	0.01	0.447
Processing losses (% of live weight)										
Intestine	5.03	4.67	4.96	4.98	4.43	5.33	4.44	4.27	0.12	0.390
Blood	4.65	3.73	3.74	3.35	3.80	3.76	3.20	3.28	0.15	0.421
Feather	5.29	5.62	5.64	5.22	5.37	5.70	5.86	5.96	0.11	0.803
Head	2.47	2.26	2.47	2.47	2.23	2.26	2.49	2.45	0.03	0.433
Separable fat	0.53	0.82	0.42	0.98	0.68	0.57	1.19	0.58	0.09	0.582
Appendages	5.21	4.80	5.39	5.32	4.94	4.91	5.10	5.07	0.08	0.693

of Fabricius was observed in the MGH0.8 and AGP groups, while the lowest weight was noted in the CON group. Similarly, diets with MGR1.0 and MG1.0 showed significantly ($p<0.05$) higher serum IgG and IL-10 levels, respectively, compared to the CON group.

Serum biochemical parameters

SGPT and SGOT are enzymes primarily located in the liver cells of broiler chickens. They serve as biomarkers to evaluate liver health and function. A diet supplemented with varying levels of fatty acid esters and an emulsifier (0.8, 1.0 g/kg) did not significantly influence ($p>0.05$) the concentrations of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase in serum (Table 05).

Histo-morphometry of Small Intestine

Morphometrical analysis of the intestine in broilers, focusing on villus height, crypt depth, intestinal wall thickness, goblet cells per field, and the villus height: crypt depth ratio at 35 days of age, is presented in Table 06. Broilers supplemented with MGH0.8 g exhibited significantly ($p<0.05$) higher villus height in the duodenum, while

Table 5: Immune response of broilers under different treatment group

Parameters	Treatments									
	CON	AGP	MG 0.8	MGR 0.8	MGH 0.8	MG1	MGR1	MGH1	SEM	*P-value*
Haemagglutination inhibition test										
Day 30	1.97	2.14	2.24	2.27	2.39	2.21	2.24	2.21	0.08	0.998
Day 42	2.04^{b}	2.21^{ab}	2.41^{ab}	2.39^{ab}	2.53^{a}	2.39^{ab}	2.37^{ab}	2.37^{ab}	0.04	0.022
Weight of lymphoid organ										
Spleen	0.14	0.11	0.09	0.13	0.13	0.14	0.10	0.11	0.00	0.227
Thymus	0.70	0.46	0.57	0.59	0.54	0.61	0.72	0.46	0.03	0.387
Bursa of fabricius	0.12^{b}	0.26^{a}	0.21^{ab}	0.17^{ab}	0.26^{a}	0.25^{a}	0.17^{ab}	0.18^{ab}	0.01	0.006
ELISA										
IgG (µg/ml)	19.48^{b}	21.27^{ab}	23.89^{ab}	23.25^{ab}	25.25^{ab}	26.57^{a}	26.63^{a}	25.27^{ab}	0.74	0.010
IL-10 (ng/l)	9.90^{b}	11.78^{ab}	14.36^{ab}	14.43^{ab}	14.49^{ab}	17.96^{a}	14.45^{ab}	15.36^{ab}	0.71	0.008

Means within the same row with different superscripts are significantly different ($p<0.05$)

Table 6: Serum biochemical parameters of broilers in different treatment groups

Parameters (IU/L)	Treatments									
	CON	AGP	MG0.8	MGR0.8	MGH0.8	MG1	MGR1	MGH1	SEM	*P-value*
SGOT	245.45	265.08	240.41	245.00	264.75	269.75	231.30	234.00	7.84	0.88
SGPT	6.11	6.41	5.98	6.70	6.08	6.50	6.86	6.40	0.37	0.99

Table 7: Histo-morphometry of small intestine in different treatment groups

Treatment	CON	AGP	MG 0.8	MGR 0.8	MGH 0.8	MG 1.0	MGR1	MGH1	SEM	*P-value*
Villus height (µm)										
Duodenum	1212.46^{c}	1311.83^{bc}	1364.27^{abc}	1398.27^{abc}	1536.83^{a}	1450.56^{ab}	1509.35^{ab}	1429.63^{ab}	27.41	0.033
Jejunum	980.00^{e}	1056.17^{de}	1123.31^{d}	1252.67^{c}	1305.73^{abc}	1338.15^{ab}	1377.76^{a}	1284.03^{bc}	29.05	0.000
Ileum	546.53^{d}	662.59^{c}	730.98^{bc}	727.15^{bc}	768.12^{ab}	843.87^{a}	849.78^{a}	811.62^{a}	21.20	0.000
Crypt depth (µm)										
Duodenum	198.96^{a}	189.87^{ab}	186.03^{abc}	183.14^{abc}	149.43^{d}	136.57^{d}	165.77^{bcd}	157.31^{cd}	5.17	0.003
Jejunum	218.10^{a}	203.42^{ab}	183.50^{bc}	186.57^{bc}	161.73^{cd}	141.64^{d}	158.33^{cd}	166.31^{cd}	5.65	0.001
Ileum	187.53^{a}	175.68^{ab}	159.11^{ab}	159.64^{ab}	152.87^{b}	149.33^{b}	155.01^{b}	156.57^{b}	3.71	0.123
Intestinal wall thickness (µm)										
Duodenum	304.95^{c}	347.57^{b}	392.71^{ab}	352.27^{ab}	398.20^{a}	391.22^{ab}	351.56^{ab}	380.31^{ab}	7.45	0.003
Jejunum	320.41^{d}	350.71^{cd}	403.43^{abc}	362.52^{bcd}	412.72^{ab}	432.40^{a}	387.02^{abc}	422.58^{a}	9.11	0.003
Ileum	336.15^{d}	380.25^{cd}	432.14^{abc}	404.24^{bcd}	467.86^{ab}	489.42^{a}	387.68^{cd}	468.07^{ab}	12.16	0.002
Goblet cells per field										
Duodenum	25.00	24.66	24.00	23.67	23.66	24.00	25.00	24.00	0.66	0.999
Jejunum	34.00	33.66	33.66	33.66	34.00	32.33	34.33	34.33	0.51	0.993
Ileum	85.33	86.66	85.33	82.33	84.33	84.00	83.00	83.66	0.59	0.743
Villous height/Crypt depth ratio										
Duodenum	6.19^{c}	7.04^{c}	7.32^{bc}	7.65^{bc}	10.34^{a}	10.62^{a}	9.14^{ab}	9.17^{ab}	0.36	0.327
Jejunum	4.51^{f}	5.21^{ef}	6.12^{de}	6.74^{cd}	8.16^{abc}	9.44^{a}	8.82^{ab}	7.82^{bc}	0.36	0.095
Ileum	2.98^{d}	3.77^{cd}	4.65^{abc}	4.56^{bc}	5.06^{ab}	5.65^{a}	5.48^{ab}	5.20^{ab}	0.19	0.000

Means within the same row with different superscripts are significantly different ($p<0.05$)

Table 8: Caecal microbiota count (log_{10} cfu/g) of broilers in different treatment groups

Caecal microbiota	Treatments									
	CON	AGP	MG0.8	MGR0.8	MGH0.8	MG1	MGR1	MGH 1.0	SEM	*P-Value*
E. coli	8.14^{a}	7.93^{ab}	7.82^{abc}	7.96^{ab}	6.9^{bc}	6.79^{c}	7.12^{abc}	7.15^{abc}	0.15	0.083
C. perfringens	7.12^{a}	6.92^{ab}	6.72^{b}	6.86^{b}	6.27^{cd}	6.18^{d}	6.44^{c}	6.27^{cd}	0.09	0.000

Means within the same row with different superscripts are significantly different ($p<0.05$)

in the jejunum and ileum, significantly ($p<0.05$) greater villus height was observed in birds supplemented with MGR1.0 compared to the CON group. Diets supplemented with MG1.0 resulted in significantly ($p<0.05$) lower crypt depth and higher VH:CD in the duodenum, jejunum, and ileum. Broilers fed diets with varying levels of fatty acid esters along with emulsifier (0.8, 1.0 g/kg feed) did not significantly ($p>0.05$) influence goblet cells per field, with values being statistically ($p>0.05$) similar across all dietary treatments. Diets containing MGH0.8 demonstrated

significantly ($p<0.05$) maximum intestinal wall thickness in the duodenum. However, in the jejunum and ileum, significantly ($p<0.05$) greater intestinal wall thickness was noted in birds supplemented with MG1.0 compared to the CON group.

Cecal microbiota count of broilers

The cecum of broiler chickens hosts a diverse microbial community that plays a crucial role in maintaining gut health and overall well-being. The supplementation of varying levels of fatty acid esters along with an emulsifier, with energy levels of 0.8 and 1.0 g/kg, on cecal microbiota (E. coli and C. perfringens) count (cfu/g) in broilers at 35 days of age is presented in Table 07. Broilers fed a basal diet supplemented with MG1.0 showed significantly ($p<0.05$) lower cecal E. coli and C. perfringens counts compared to the CON and AGP groups.

Discussion

Growth Performance

The improved nutrient utilization observed in the present study is consistent with previous reports demonstrating beneficial effects of fatty acid esters and emulsifiers on fat and protein digestibility in broilers. Alpha-monolaurin and dietary emulsifiers have been shown to improve crude fat utilization and ileal digestibility of crude protein and fat (Saleh et al., 2021; Shahid et al., 2021; Ahmadi-Sefat et al., 2022). These effects may be attributed to enhanced amino acid digestibility, improved triglyceride lipolysis, and increased micelle formation, which facilitate lipid digestion and absorption, particularly in young broilers with limited lipase activity (Amer et al., 2020; Cho et al., 2012). The improved growth performance and feed efficiency observed in the present study are also in agreement with earlier studies reporting enhanced body weight, weight gain, average daily gain, feed conversion ratio, and production efficiency following supplementation with alpha-monolaurin, monoglycerides, GML, and emulsifier blends, particularly in reduced-energy diets (Saleh et al., 2021; Liu et al., 2021; Ahmadi-Sefat et al., 2022; Fortuoso et al., 2019; Ghazalah et al., 2021; Mustafa, 2019; Kong et al., 2021; Shahid et al., 2021).

The superior economic efficiency and lower feed cost per kilogram of weight gain in the MGH0.8 group indicate the potential of fatty acid esters and emulsifiers as cost-effective alternatives to conventional growth-promoting strategies. The enhanced performance may be associated with the antimicrobial and anti-inflammatory properties of fatty acid esters, along with the ability of medium-chain fatty acids to provide energy to intestinal epithelial cells, support intestinal integrity, and improve nutrient absorption (Hwang et al., 2012; Khosravinia, 2015). The greater jejunal villus length and improved villus height-to-crypt ratio in the duodenum and ileum may have further contributed to enhanced nutrient utilization and feed efficiency. However, the absence of significant effects on meat fat and protein content is consistent with previous findings, suggesting that these supplements primarily improve growth performance, nutrient utilization, and intestinal function rather than meat composition (Ghazalah et al., 2021).

Immunological Response

The improved immune response observed in the present study is consistent with previous reports demonstrating the immunomodulatory potential of emulsifiers and medium-chain fatty acid derivatives. Dietary herbal emulsifier and alpha-monolaurin supplementation enhanced specific antibody responses against Newcastle disease virus (NDV) in broilers, particularly under energy-restricted feeding conditions (Gole et al., 2022; Saleh et al., 2021). Similarly, the decline in vaccine-specific immunity associated with reduced dietary energy may be alleviated by emulsifier supplementation (Medagoda et al., 2021; Wangkahart et al., 2022). These effects may be mediated through modulation of gut microbiota and reduction of oxidative stress (Liu et al., 2013). In the present study, the increased relative weight of the bursa of fabricius may further indicate enhanced humoral immune development, although previous studies have reported variable effects of emulsifiers on immune organ weights, with no significant changes in spleen and thymus weights (Lonkar et al., 2017; Allahyari-Bake & Jahanian, 2017).

The immunostimulatory effects of fatty acid esters and related compounds may be attributed to their antimicrobial, antiviral, and anti-inflammatory properties. Monolaurin can disrupt the lipid and phospholipid structures of pathogen envelopes, thereby exerting virucidal and bactericidal effects. In addition, GML supplementation has been associated with increased IL-10 and serum IgG concentrations, indicating enhanced anti-inflammatory and humoral immune responses (Amer et al., 2020; Kong et al., 2021). Medium-chain fatty acids may also regulate immune-cell signaling through GPR84, modulating cytokine responses and reducing LPS-induced pro-inflammatory mediators while enhancing IL-10 production. Collectively, these mechanisms may explain the improved immune response and development of immune organs observed in the present study.

Histomorphometry of Intestine

The present findings are consistent with Amer et al. (2020), who reported increased duodenal and jejunal villus height and jejunal muscle thickness with 0.1% GML supplementation, along with reduced jejunal crypt depth at 0.3 and 0.5% GML. Increased villus height enhances the absorptive surface, enzyme activity, and nutrient uptake, while MCFA and related glycerides can be directly absorbed by enterocytes to provide energy and support intestinal

development. Similarly, Kong et al. (2021) observed reduced crypt depth and an increased VH:CD ratio with 300–1200 mg/kg GML supplementation, indicating improved mucosal turnover and digestive and absorptive capacity. The beneficial effects of GML on intestinal integrity may also be associated with its anti-inflammatory activity, including suppression of pro-inflammatory cytokines and downregulation of the TLR4/NF-κB pathway. Likewise, Ghazalah et al. (2021) reported increased villus height and reduced crypt depth following LEX supplementation in broilers fed low-energy diets, possibly due to improved fat emulsification, smaller emulsion droplet formation, faster micelle formation, and reduced ileal digesta viscosity.

Caecal Microbiota count of Broilers

Basit et al. (2020) reported that supplementation with *Persicaria odorata* leaf meal (8 g/kg) and Piper betle leaf meal (PBLM; 4 g/kg), along with haloquinol and antibiotics, reduced caecal *E. coli* counts, while PBLM also reduced *C. perfringens* populations in broilers. Similarly, the reduced caecal microbial counts observed in the present study may be attributed to the antimicrobial activities of bioactive compounds such as quercetin and eugenol (Wang et al., 2018). Eugenol acts against both Gram-negative *E. coli* and Gram-positive *C. perfringens* by increasing cell wall and membrane permeability and disrupting the cytoplasmic membrane, whereas quercetin exhibits bacteriostatic activity against *E. coli* (Sharma et al., 2014).

Conclusions

Fatty acid esters with emulsifier improved broiler performance, nutrient utilization, immunity, and intestinal morphology, while maintaining carcass traits and biochemical health. MGH0.8 was the most economical treatment and showed potential as a cost-effective alternative to antibiotic growth promoters.

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References

1) Abreu R, Semedo-Lemsaddek T, Cunha E, Tavares L, Oliveira M, 2023. Antimicrobial Drug Resistance in Poultry Production: Current Status and Innovative Strategies for Bacterial Control. Microorganisms, 11(4): 953. doi:10.3390/microorganisms11040953.
2) Ahmadi-Sefat AA, Taherpour K, Ghasemi HA, Gharaei MA, Shirzadi H, Rostami F. 2022. 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. Poultry Science, 101(11): 102145. doi: 10.1016/j.psj.2022.102145.
3) Allahyari-Bake S, Jahanian R. 2017. Effects of dietary fat source and supplemental lysophosphatidylcholine on performance, immune responses, and ileal nutrient digestibility in broilers fed corn/soybean meal-or corn/wheat/soybean meal-based diets. Poultry Science, 96(5): 1149-1158. doi: 10.3382/ps/pew330.
4) Amer SA, Nasser AA, Al-Khalaifah HS, AlSadek DM, Abdel Fattah DM, Roushdy EM, Sherief WR, Farag MF, Altohamy DE, Abdel-Wareth AA, Metwally AE. 2020. Effect of dietary medium-chain α-monoglycerides on the growth performance, intestinal histomorphology, amino acid digestibility, and broiler chickens’ blood biochemical parameters. Animals, 11(1): 57. doi: 10.3390/ani11010057.
5) AOAC, 2012. Official methods of Analysis, 19th Edn., Association of Analytical Chemists, Washington, D.C.
6) Cho JH, Zhao P, Kim IH. 2012. Effects of emulsifier and multi-enzyme in different energy densitydiet on growth performance, blood profiles, and relative organ weight in broiler chickens. The Journal of Agricultural Science, 4(10): 161. doi: 10.5539/jas.v4n10p161.
7) Drury RAB and Wallington EA. 1980. Carleton’s Histological Technique, 5th Edn., Oxford University Press, pp 99-113.
8) Duncan DB. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42. doi:10.2307/3001478.
9) Fortuoso BF, Dos Reis JH, Gebert RR, Barreta M, Griss LG, Casagrande RA, de Cristo TG, Santiani F, Campigotto G, Rampazzo L, Stefani LM, Da Silva AS. 2019. Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: Impact on health, performance and meat quality. Journal of Microbial Pathogenesis, 129: 161-167. doi: 10.1016/j.micpath.2019.02.005.

10) Galli GM, Aniecevski E, Petrolli TG, da Rosa G, Boiago MM, Simoes CA, Wagner RPR, Copetti PM, Morsch VM, Araujo DN, Marcon H, Da Silva AS. 2021. Growth performance and meat quality of broilers fed with microencapsulated organic acids. Animal Feed Science and Technology, 271: 114706. doi: 10.1016/j.anifeedsci.2020.114706.
11) Ghazalah A, Abd-Elsamee M, Ibrahim M, Abdelgayed SS, Abdelkader M, Gonzalez-Sanchez D, Wealleans A. 2021. Effects of a combination of lysolecithin, synthetic emulsifier, and monoglycerides on growth performance, intestinal morphology, and selected carcass traits in broilers fed low-energy diets. Animals, 11: 3037. doi: 10.3390/ani11113037.
12) Gobezie E 2022. Effect of Neem (Azadirachta indica) leaf powder on the growth performance and carcass quality of Broiler Chicken – A Review. Journal of Livestock Science 13: 152-158 doi. 10.33259/JLivestSci.2022.152-158
13) Gole M, Manwar S, Khose K, Rathod P, Kumar D, Ganguly RK. 2022. Efficacy evaluation of a poultry feed emulsifier in broiler chicken. The Pharma Innovation Journal, 11(1): 11-19.
14) Hubbard SAS. 1999. Broiler management guide, Hubbard SAS, Quintin, France.
15) Hwang D, Huang S, Rutkowsky JM, Snodgrass RG, Ono-Moore KD, Schneider DA, Newman JW, Adams SH. 2012. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. The Federation of American Societies for Experimental Biology, 26(1): 364. doi: 10.1096/fasebj.26.1_supplement.364.8.
16) Khosravinia H. 2015. Effect of dietary supplementation of medium-chain fatty acids on growth performance and prevalence of carcass defects in broiler chickens raised in different stocking densities. Journal of Applied Poultry Research, 24(1): 1-9. doi: 10.3382/japr/pfu001.
17) Kong L, Wang Z, Xiao C, Zhu Q., Song Z. 2021. Glycerol monolaurate ameliorated intestinal barrier and immunity in broilers by regulating intestinal inflammation, antioxidant balance, and intestinal microbiota. Frontiers in Immunology 12: 713485. doi: 10.3389/fimmu.2021.713485.
18) Lan J, Chen G, Cao G, Tang J, Li Q, Zhang B, Yang C. 2021. Effects of α-glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens. Poultry Science, 100(3): 100875. doi: 10.1016/j.psj.2020.11.052.
19) Liu T, Li C, Zhong H, Feng F. 2021. Dietary medium-chain α-monoglycerides increase BW, feed intake, and carcass yield in broilers with muscle composition alteration. Poultry Science, 100(1): 186-195. doi: 10.1016/j.psj.2020.09.056.
20) Liu XC, Yang ZB, Yang WR, Jiang SZ, Zhang GG. 2013. In vitro study of effects of emulsified oil on broiler feed quality. Animal Science Journal, 84(3): 231-237. doi: 10.1111/j.1740-0929.2012.01056.x.
21) Lonkar VD, Kadam AS, Ranade AS, Mote CS. 2017. Effect of Emulsifier on Carcass Traits, Serum Biochemical and Histopathological Changes in Liver and Kidney of Broiler Chicken Fed with Low Energy Diet. The Journal of Bombay Veterinary College, 24(1): 32.
22) Markey B, Leonard F, Archambault M, Cullinane A, Maguire D, Edwards R, Hewat C. 2013. Clinical Veterinary Microbiology, 2nd Edn., Mosby Elsevier, London, pp 215-239.
23) Medagoda N, Kim MG, Gunathilaka BE, Park SH, Lee KJ. 2022. Effect of total replacement of fish oil with tallow and emulsifier in diet on growth, feed utilization, and immunity of olive flounder (Paralichthys olivaceus). Journal of the World Aquaculture Society, 53(2): 558-571. doi: 10.1111/jwas.12835.
24) Mueller EA and Schlievert PM. 2015. Non-aqueous glycerol monolaurate gel exhibits antibacterial and anti-biofilm activity against Gram-positive and Gram-negative pathogens. PloS one 10(3): e0120280. doi: 10.1371/journal.pone.0120280.
25) Mustafa NG. 2019. Biochemical trails associated with different doses of alpha-monolaurin in chicks. Advances in Veterinary & Animal Sciences, 7(3): 187-192. doi: 10.17582/journal.aavs/2019/7.3.187.192.
26) Office International des Epizooties. 2004. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Office International des Epizooties, Paris, pp 258–269.
27) Oketch EO, Wickramasuriya SS, Oh S, Choi JS, Heo JM. 2023. Physiology of lipid digestion and absorption in poultry: An updated review on the supplementation of exogenous emulsifiers in broiler diets. Journal of Animal Physiology & Animal Nutrition, 107(6): 1429-1443. doi: 10.1111/jpn.13859.
28) Prem Kumar R, Venkateswarlu S, Srinivasa Rao D and Padmaja K 2026. Antioxidant status, serum biochemical profile and Duodenal morphometry of broilers supplemented with Spirulina (Arthrospira) and exogenous protease enzyme. Journal of Livestock Science 17: 407-412 doi. 10.33259/JLivestSci.2026.407-412
29) Rarokar NR, Menghani S, Kerzare D, Khedekar PB. 2017. Progress in synthesis of monoglycerides for use in food and pharmaceuticals. Journal of Experimental Food Chemistry, 3(3): 13-19. doi: 10.4172/2472-0542.1000128.

30) Reitman S and Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of clinical Pathology, 28(1), 56-63. doi: 10.1093/ajcp/28.1.56.
31) Saleh AA, El-Gharabawy B, Hassan A, Badawi N, Eid Y, Selim S, Shukry M, Dawood M. 2021. Effect of dietary inclusion of alpha-monolaurin on the growth performance, lipid peroxidation, and immunity response in broilers. Sustainability, 13(9): 5231. doi: 10.3390/su13095231.
32) Schlievert PM, Kilgore SH, Seo KS, Leung DY. 2019. Glycerol monolaurate contributes to the antimicrobial and anti-inflammatory activity of human milk. Scientific Reports, 9(1): 14550.
33) Seleem D, Chen E, Benso B, Pardi V, Murata RM. 2016. *In vitro* evaluation of antifungal activity of monolaurin against Candida albicans biofilms. Peer Journal of Life and Environmental Sciences, 4: e2148. doi: 10.7717/peerj.2148.
34) Shahid I, Sharif M, Yousaf M, Ahmad F, Virk MR, Bilal MQ, Anwar U, Ali A, Hussain M, Chishti MFA, Rahman MA. 2021. Effect of exogenous emulsifier (lyso-phospholipid) supplementation in the broiler diet, on the feed intake and growth performance during grower phase. Brazilian Journal of Poultry Sciences, 23(1): eRBCA-2020. doi: 10.1590/1806-9061-2020-1354.
35) Sharma G, Raturi K, Dang S, Gupta S, Gabrani R. 2014. Combinatorial antimicrobial effect of curcumin with selected phytochemicals on Staphylococcus epidermidis. Journal of Asian Natural Products Research, 16(5): 535-541. doi: 10.1080/10286020.2014.911289
36) Skrivanova E, Prazakova S, Benada O, Hovorkova P, Marounek M. 2014. Susceptibility of Escherichia coli and Clostridium perfringens to sucrose monoesters of capric and lauric acid. Czech Journal of Animal Science, 59(8): 374-380.
37) Snedecor GW, Cochran WG. 1994. Statistical Method, 8th Edn., The lowa State University Press, Ames, Lowa, USA.
38) Tan H, Zulkifli I, Farjam AS, Goh YM, Croes E, Partha SK, Tee AK. 2016. Effect of exogenous emulsifier on growth performance, fat digestibility, apparent metabolisable energy in broiler chickens. Journal of Biochemistry Microbiology & Biotechnology, 4(1): 7-10.
39) Tsugkieva VB, Tsugkiev BG, Dzantieva LB, Kokoeva AT, Tokhtieva LKh, Shabanova IA, Tokhtieva EA 2021. Effect of feeding Yeast obtained from Sakhalin Buckwheat on the growth of broiler chickens. Journal of Livestock Science 12: 71-75 doi. 10.33259/JLivestSci.2021.71-75
40) Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, Xiao F, Li Y, Yin W. 2018. Bacteriostatic effect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism *in vitro*. Journal of Food Protection, 81(1): 68-78. doi: 10.4315/0362-028X.JFP-17-214.
41) Wangkahart E, Bruneel B, Wisetsri T, Nontasan S, Martin SA, Chantiratikul A. 2022. Interactive effects of dietary lipid and nutritional emulsifier supplementation on growth, chemical composition, immune response and lipid metabolism of juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture, 546: 737341. doi:10.1016/j.aquaculture.2021.737341.
42) Yoon BK, Jackman JA, Valle-Gonzalez ER, Cho NJ. 2018. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. International Journal of Molecular Science, 19(4): 1114. doi: 10.3390/ijms19041114.