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Optimising Growth, Immunity, and Gene Expression in Broiler Chickens Through Dietary Threonine Levels and Oil Inclusion

Mohamed I. El-Katcha[1] | Mosaad A. Soltan[1] | Heba I. Ghamry[2] | Abeer F. El-Nahas[3] | Laila A. Al-Shuraym[4] | Ostan Mihaela[5] | Rada Olga[5] | Rasha E. Azab[6] | Ahmed Abdeen[7] | Mustafa Shukry[8] 🄳 | Set A. El-Shobokshy[1]

[1]Nutrition and Veterinary Clinical Nutrition Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt | [2]Nutrition and Food Science, Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia | [3]Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt | [4]Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia | [5]Department of Biology and Plant Protection, Faculty of Agriculture, University of Life Sciences "King Michael I" from Timișoara, Timișoara, Romania | [6]Department of Physiology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt | [7]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt | [8]Department of Physiology, Faculty of Veterinary Medicine, University of Kafrelsheikh, Kafrelsheikh, Egypt

Correspondence: Mustafa Shukry (mostafa.ataa@vet.kfs.edu.eg) | Rada Olga (olga_rada@usvt.ro)

ABSTRACT
Background: The inclusion of synthetic amino acids in poultry nutrition plays a crucial role in both enhancing the synthesis of immunoglobulins and elevating the overall comprehensiveness of the amino acid profile.
Objectives: This research examined the effects of consuming threonine (Thr) in various forms levels with low or high oil on broiler chickens' growth and immunity.
Methods: We investigate the growth performance, feed efficiency, immune response, intestinal morphology, absorptive capacity, and expression of some genes related to the feed intake (Pro-opiomelanocortin [POMC]), fatty acid synthesis (Acetyl-CoA Carboxylase [ACC]), immunity (lipopolysaccharide-induced tumour necrosis like alpha factor [LITAF]), and heat shock protein 70 (HSP70). Eight groups of chicks were used, including four dietary Thr levels (100%, 115%, 130%, or 145%) with two oil levels (mixture of sunflower 50% and soybean oils 50%): (control) and high.
Results: The higher dietary Thr level (145%) with high oil inclusion significantly increased ACC and POMC gene expression, resulting in the lowest feed intake, body weight gain (BWG), and liver fat content. Combining high oil with 115% Thr was the optimum for the broilers. The birds have significant ($p \leq .05$) growth performance, immune parameters, and intestinal health, as well as the lowest expression of ACC, POMC, HSP70, and LITAF, which was reflected in better feed conversion ratio and lower incidence of fatty liver, thermo-resistance, and immune status of the birds.
Conclusions: The combination of high oil and 115% Thr levels optimises broiler health and productivity, enhancing growth, immune function, and gut health. This diet lowers the expression of genes associated with fatty liver and stress, leading to better

feed efficiency, thermo-resistance, and overall well-being. Adopting these dietary adjustments can improve broiler performance and economic viability in poultry farming by enhancing essential productivity metrics.

1 | Introduction

Different nutrients affect animal metabolism and immune system function (Wolowczuk et al. 2008). Protein and oils are essential nutrients that achieve the best growth and immunity. However, the animal's susceptibility to various infectious diseases under commercial conditions may increase its nutrient requirements to enhance immunity (Leeson 2006). Supplementations of synthetic amino acids (AAs) in poultry diets are essential for immunoglobulin synthesis and also improve the completeness of the AA profile (Moghaddam et al. 2011). Threonine (Thr) is an essential AA for protein synthesis and enhances poultry immunity (Li et al. 2007). It serves as a precursor to glycine and serine, which are involved in immune responses. It is required in mucin protein production of the gastrointestinal tract to maintain intestinal immune function (Pelaseyed et al. 2014), inhibit apoptosis (Sheng et al. 2011), stimulate lymphocyte proliferation, and enhance antibody production (Rosado et al. 2015). It improved the ability of broilers to heat stress (Lemme 2001). This improvement is largely attributed to the oils' fatty acid profiles, which can influence cellular membrane fluidity and integrity, thereby supporting better metabolic heat regulation and reducing oxidative stress in the birds. Furthermore, the supplementation of Thr enhanced the intestinal morphology and the density of the goblet (Chen et al. 2017). Again, Thr is an essential component of γ-globulin. Therefore, its deficiency reduces the antibody production (Li et al. 2007). Several studies on the chicken have investigated the effects of supplementing with Thr on the expression of immune-related genes. Increasing Thr levels enhances antioxidant and immune capacity, while maintaining overall body homeostasis (Ji et al. 2019). Furthermore, arginine and Thr were shown to boost growth-related gene expression, and Thr along with Met+Cys influenced immune gene expression in broiler chickens (Bhanja et al. 2014). Additionally, Thr supplementation was effective in mitigating LPS-induced inflammatory responses and protecting the intestinal barrier in young broilers (Chen et al. 2018)

High levels of Thr have been shown to decrease the mRNA levels of pro-inflammatory mediators such as IL-10, IL-12, IFN-γ, and IL-1β. This reduction in pro-inflammatory mediators indicates an improvement in gut health. Furthermore, increased mucin-2 (MUC2) mRNA expression has been observed, suggesting enhanced mucin production, which plays a role in maintaining intestinal barrier function (Chen et al. 2018). By increasing the expression of TNF-α in the spleen tissue, the in ovo Thr injection of chicken embryos improves the birds' immunity (Bhanja, Hotowy, and Mehra 2015). Furthermore, as a supplement, it reduces belly fat in birds by inhibiting hepatic FAS mRNA expression (Wu, Fang, and Guo 2011). Broiler diets that include oil offer numerous advantages. It improves feed efficiency and growth performance, decreases marketing age, minimises heat increment during heat stress, reduces the food passage time along the gastrointestinal tract, and increases digestibility and absorption. Also, it serves as a spring of essential fatty acids that

are important for the immune system (Jalali, Rabiei, and Kheiri 2015). Several genes are known to affect lipid metabolism and immunity. Among them are genes encoding acetyl-CoA carboxylase enzymes, essential regulators of the hepatic lipid metabolic transitions (Saggerson 2008). Pro-opiomelanocortin (POMC) is a hypothalamic neuropeptide expressed mainly in the anterior and intermediate lobes of the pituitary and the hypothalamus. It produces a corticotropin-releasing hormone that promotes anorexia (decreases feed intake and raises catabolic processes) in mammals and birds (Richards 2003). Hepatic lipopolysaccharide-induced tumour necrosis alpha factor (LITAF) is a potent inducer of macrophages and monocytes, causing the creation of TNF-alpha (Wanidworanun and Strober 1993). A cellular component known as heat shock protein 70 (HSP70) protects cells from oxidative and thermal stress and plays a crucial role in protein folding (Morano 2007). Dietary components such as Thr and oil significantly influence the expression and activity of several key genes involved in lipid metabolism and immune function. Among these, acetyl-CoA carboxylase (ACC) enzymes are crucial as they regulate fatty acid synthesis and oxidation, processes that are essential for managing lipid levels in the liver. Thr is vital for protein synthesis and can affect the production of enzymes like ACC, impacting lipid metabolism (Yu et al. 2019). Additionally, dietary oils, rich in fatty acids, directly influence the lipid composition within cells, potentially altering the activity of ACC. Thr, an essential AA, may impact the expression of POMC, as AAs are known to influence hormone synthesis, thereby affecting how animals respond to hunger and energy storage (Xiao and Guo 2022). The presence of specific fatty acids from dietary oils can modulate the immune response by affecting the expression of genes like LITAF, enhancing the body's ability to respond to infections and inflammation (Wanidworanun and Strober 1993). Both Thr and dietary oils can affect the expression of HSP70, with implications for cellular protection mechanisms under stress conditions, such as heat and oxidative stress (Moura et al. 2018). However, no data are available on the effect of the dietary combination of Thr and oil on the expression of these genes in chickens. So, in this study, we aim to examine how different levels of dietary Thr and different amounts of dietary oil affect various blood parameters, growth, immune response, intestinal histopathology, and gene expression in broiler chickens. These parameters pertain to feed intake, immunity, and lipid metabolism

2 | Materials and Methods

2.1 | Housing and Care for Birds

In this study, 400 avian non-sexed chicks aged 1 day were utilised. The chicks were sourced from a private hatchery in Behera governorate, Egypt. To ensure equal distribution, the chicks were divided into eight groups of similar size, each consisting of 50 chicks, five replicates per each group, and each replicate has 10 birds. The birds were provided with optimal housing conditions

and received regular vaccination protocols. Chicks were housed in a clean well-ventilated room previously fumigated with formalin and potassium permanganate. The room was provided with electric heaters to adjust the environmental temperature according to the age of the birds. Each pen was provided by suitable feeder and waterer. Feeds and water were supplied ad libitum. Throughout the experiment, all handling of the birds adhered to the ethical guidelines set by the Committee on the Ethics of Animal Experiments at Alexandria University, Egypt (Alex-IACUC/0014/2023) and accordance with the ARRIVE guidelines to ensure the highest standards of experimental reporting and reproducibility.

2.2 | Program for Feeding and Experimental Design

During the first 2 weeks of the experiment, the chicks were fed a formulated ration consisting of a starter diet. From weeks 3—4, they were switched to a grower diet; during weeks 5–6, they were provided with a finisher diet. The birds were allocated into groups randomly, resulting in eight groups, each with five replicates of 10 birds. Groups 1, 2, 3, and 4 were given diets containing four levels of graded Thr (100%, 115%, 130%, or 145% of the avian catalogue recommendation). These diets had a low oil content (control oil %). On the other hand, Groups 5, 6, 7, and 8 were fed diets with the same four levels of Thr but with an additional 2% oil compared to the control diet. This feeding regimen was maintained until the birds reached the marketing age and were ready for slaughter.

The experimental basal diets used for the starter, grower, and finisher stages, containing 100% of the avian catalogue recommendation, had specific ingredient compositions, which can be found in Table 1. To create the other three experimental diets with graded Thr concentrations (115%, 130%, and 145% of the avian catalogue recommendation), crystalline L-Thr (98.5% Thr) was added to the basal diet accordingly. We gave the control group the standard Thr requirement which represented 100% then added 15%, 30%, and 45% extra than the standard for other groups with two levels of oil: 2% as the standard and 2% extra to determine the best level of Thr and oil in broiler chicken's diet and also determine the effects of Thr as the lipotropic factor with high energy diet.

2.3 | Growth Performance

The initial body weight of each bird was measured and verified. The birds' weekly feed intake, body weight, and weight gain were carefully documented throughout the experiment. Several performance indicators, including the protein efficiency ratio (PER), feed conversion ratio (FCR), performance index (PI), and efficiency of energy utilisation (EEU), were calculated based on the recorded data.

Protein efficiency ratio (PER):

PER = Weight gain (g)/Protein intake (g)

Feed conversion ratio (FCR):

FCR = Feed intake (g)/Weight gain (g)

Performance index (PI):

PI = Weight gain (g)/(Days of the experiment × Initial body weight (g)) × 100

Efficiency of energy utilisation (EEU):

EEU = Weight gain (g)/Energy intake (kcal)

2.4 | The Chemical Composition of the Feed and Liver

The moisture content of the feed and liver samples was regulated by subjecting them to oven-drying at 105°C for 8 h, following the procedure outlined in Reynolds (1989). Ash content was decided by incinerating the samples at 550°C overnight. The crude protein content was determined using the Kjeldahl method, as labelled by Randhir and Pradhan (1981). The ether extract (EE) content was detected using the technique developed by Bligh and Dyer (1963) and Hanson and Oll (1963). EE is determined by homogenisation of wet foods with an equal amount of methanol and chloroform to extract fat. Weigh the prepared sample (Ws) into a 120-mL centrifuge bottle. Add 9 mL of distilled water, 20 mL of methanol, and 10 mL of chloroform and macerate for 2 min then add further 10 mL of chloroform. Macerate for 2 min then add 10 mL of distilled water and macerate for 30 s. Centrifuge the mixture for 10 min at 2000–2500 rpm. Draw off the lower chloroform layer containing fat without disturbing the supernatant layers by using a syringe. Filter it through a coarse filter paper. The clear filtrate is transferred into a dry previously weighed beaker (WB). Evaporate the chloroform to dryness in water bath at 75°C and complete drying in an oven at 105°C (WF). EE Wt (g) = WF − WB. EE% = [EE Wt (g)/Ws (g)] × 100. Ion-exchange chromatography was employed to determine the levels of Thr and lysine AAs in the feed samples. This analysis used equipment from Hitachi, Inc., based in Tokyo, Japan. To analyse AA, samples were ground to pass a 0.5-mm screen; feed samples as well as bag residues were acid-hydrolysed with 6N phenol-HCl for 24 h at 110oC (Miller 2004), and AA concentrations of the hydrolysates were determined by the isotope dilution method (Calder et al. 1999).

Briefly, 2 mL of the hydrolysate was diluted with 3 mL of ultrapure water, and 1 mL of this solution was then combined with 200 μL of a mixture of labelled AA (13C and 15N AA isotope standards; CDN Isotopes, Pointe-Claire, Quebec; Cambridge Isotope Laboratories Inc., Andover, MA, USA), which served as an internal standard. The solution was eluted through a poly-prep chromatography column (resin 100–200 mesh H; Bio-Rad, Hercules, CA), then derivatised with N-(tert-butyl dimethylsilyl)-N-methyl trifluoroacetamide and dimethyl formamide 1:1 (394882, 27.0547; Sigma-Aldrich) according to the method of Calder and Smith (1988). AAs were quantified using GC-MS (Hewlett-Packard Model GC6890-MS5973; Agilent Technologies, Wilmington, DE, USA) and a mass selective detector (Hewlett-Packard, Palo Alto, CA, USA). The AA threonine (Thr) and lysine were analysed separately by subjecting the samples to performic acid oxidation, followed by HCl hydrolysis (Miller 2004); these two AA were analysed

TABLE 1 | Ingredient composition of the used basal diet (100% Thr of avian catalogue recommendation).

Period Ingredient	Starter (1–14 days old)		Grower (15–28 days old)		Finisher (29–42 days old)	
	Control oil%	2% Extra oil	Control oil%	2% Extra oil	Control oil%	2% Extra oil
Yellow corn grain (7.8% CP)	53.05	49.55	58.05	54.55	62.10	58.31
Soybean meal (42.9% CP)	33.20	36.70	29.50	33.00	26.70	30.00
Corn gluten (59.2% CP)	8.00	6.00	6.50	4.50	5.00	3.50
Vegetable oil[a]	2.00	4.00	2.00	4.00	2.50	4.50
MCP[b]	1.10	1.10	1.10	1.10	1.10	1.10
Limestone[c]	1.80	1.80	2.00	2.00	1.70	1.70
Lysine[d]	0.05	0.05	0.05	0.05	0.05	0.05
DL-methionine[e]	0.10	0.10	0.10	0.10	0.10	0.10
Choline[f]	0.05	0.05	0.05	0.05	0.05	0.05
Mycotoxin adsorpant[g]	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix (mineral and vitamin)[h]	0.30	0.30	0.30	0.30	0.30	0.30
Threonine[i]	—	—	—	—	0.05	0.04
Chemical composition						
Moisture%	11.8	11.7	11.4	11.3	11.0	10.8
Ash%	5.7	6.2	5.5	6.8	5.4	6.3
Crude protein%	23.1	23.01	21.1	21.3	19.1	19.6
EE%	6.3	8.3	5.9	7.5	5.8	7.7
Calcium%*	0.95	0.96	1.00	1.01	0.87	0.88
Phosphorus%*	0.45	0.45	0.44	0.44	0.43	0.43
Methionine%*	0.53	0.52	0.498	0.486	0.46	0.46
Lysine%*	1.28	1.29	1.1	1.2	1.03	1.1
Lysine%	1.132	1.110	1.014	0.996	0.993	0.999
Threonine*	0.88	0.89	0.805	0.815	0.79	0.79
Threonine%	0.863	0.873	0.762	0.802	0.722	0.772
ME Kcal/kg diet*	3068.3	3164.8	3124.05	3190.6	3184.8	3254.9
Calorie/protein ratio*	132.8	136.7	148.6	151.4	165.4	167.0

[a]Mixture of sunflower and soybean oils.

[b]Mono calcium phosphate: contains 21% phosphorus and 16% calcium.

[c]Lime stone contains 37% calcium and locally produced.

[d]Lysine 87% produced by Archer Daniels method company De Caur LL. Made in USA.

[e]DL-methionine produced by Evoink Co. Guaranteed analysis 99.5% DL-methionine.

[f]Choline: choline chloride 60% with vegetable carrier (corn powder) produced by Shandong Pharmaceutical Co. China.

[g]Beta-2-x produced by Egavet Co.

[h]Hero mix (Hero pharm, Cairo, Egypt). Composition (per 3 kg): vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

[i]Crystalline L-Thr (98.5% Thr, PT. Cheil Jedang, Indonesia).

*Calculated according to the feed composition tables given in NRC (1994).

with a Biochrom 20 AA analyser (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.5 | Leukocytic Counts and Phagocytosis

On the 42nd day of the experiment, ~1.5 mL of blood samples were collected from seven birds in each replicate (35 birds/group). The blood samples were collected in a tube containing heparin and stored at +4°C until the analysis of haematological parameters. These samples assessed the blood count, differential leukocytic (white blood cells), phagocytic activity, and phagocytic index. The count of white blood cells (WBCs) was established using the method depicted by (Benjamin 1986). A blood film was prepared, and the differential leukocytic count was conducted following the technique outlined by Lucky and Lucký (1977). To evaluate phagocytic activity and phagocytic index, the methods labelled by Kawahara, Ueda, and Nomura (1991) were employed.

2.6 | Lymphoid Organs Weight

On the 42nd day, seven birds in each replicate (35 birds/group) were randomly selected from each treatment and fasted for six hours. We measured the relative weight of the spleen, bursa, and thymus gland, organs connected to the immune system. We also made sure the birds were thoroughly bloodied.

2.7 | Assessment of Blood Parameters

Commercial kits (Reactivos GPL Chemelex, S.A. Pol. Ind. Can Castells. C/Industria 113, Nau J 08420 Canovelles-Barcelona) were used to estimate the amounts of serum lipids (cholesterol, triglyceride, HDL, LDL, and VLDL) using an auto-analyser (HITACHI 902 automated auto-analyser; Roche Diagnostics, Basel, Switzerland).

2.8 | Determination of Intestinal Morphology and Absorptive Capacity

On day 42, two chickens from each replicate were selected for slaughter. A 2.5-cm segment of ileal tissue sampled at the midpoint of the region extending from Meckel's diverticulum to the ileocecal junction and a 2.5-cm segment of jejunum middle tissue were collected and submersed in 10% neutral-buffered formalin for 24 h to fix the tissue. After fixation, soaked samples were rinsed several times in absolute alcohol, and then embedded in paraffin. Serial 6-μm longitudinal sections were cut on Leica Rotary Microtome (RM 2145; Leica Microsystems, Wetzlar, Germany) and placed on glass slides. Then, slides routinely stained with Gill's haematoxylin and eosin (H&E). For the histochemical evaluation of gut mucins, other representative sections were stained with Periodic acid Schiff for the detection of neutral mucins (Law et al. 2007). Neutral and acid mucin staining neutral mucin was measured by staining 6-μm sections with periodic acid-schiff (PAS) (Uni, Smirnov, and Sklan 2003). Briefly, procedure steps consist of (i) deparaffinise and hydrate to eliminate the contribution of sialic acid residues before PAS staining; (ii) oxidise in 0.5% periodic acid solution for 15 min; (iii) rinse in distilled water; (iv) immerse in Schiff reagent for 30 min (sections become light pink colour during this step); (v) wash in warm water for 10 min (immediately sections turn dark pink colour); and (vi) dehydrate with ethanol and mount in glass slide. The number of PAS positive (PAS+) along the villi was counted by light microscopy.

Histomorphometric analysis was performed on H&E-stained tissue sections. The parameters measured were as follows: villus height (measured from the tip of the villus to the villus-crypt junction), crypt depth (measured from the crypt-villus junction to the base of the crypt), and villus surface area $[(\pi \times mh \times h) + (\pi \times mh/2)2]$, where mh is the width at the midvillus height and h is villus height (Law et al. 2007). Villi length and width were measured from five villi per bird, and only complete, vertically oriented villi were measured. Goblet cell counts were taken from the same five villi per bird, and the average value was used. The density of goblet cells was calculated as the number of goblet cells per unit of surface area (mm^2). The morphometric study was assessed by the examination of villus length, width, and crypt depth. The measurements were done using a computerised image analysis system (ImageJ software, Bethesda, MD, USA).

2.9 | Expression Analysis of Genes Related to Feed Intake, Immunity, and Lipid Metabolism

The hypothalamus and liver tissues were collected from 10 birds per group and stored in clean tubes at −80°C until further use. RNA extraction was performed using the TRIsure Kit from the bio line. To ensure RNA quality for gene expression studies, RNA is first isolated, and its concentration was measured via spectrophotometry, checking for an optimal A260/A280 ratio near 2.0. RNA integrity is further assessed through agarose gel electrophoresis. For reliable gene expression analysis, housekeeping genes 18S rRNA are chosen due to their constant presence and stable expression across various tissues and conditions. The stability of 18S rRNA under specific experimental conditions is validated using the geNorm software.

Subsequently, the cDNA kit was synthesised following the manufacturer's protocol using the iNtRON Biotechnology kit. Gene-specific primer sequences for POMC, LITAF, ACC, and HS70 can be found in Table 2. The Stratagene MX3000P real-time PCR system was employed to assess gene expression. The SensiFast SYBR Lo-Rox kit from Bioline was used for the PCR reactions. The thermal cycler conditions included an initial denaturation step at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 2 s and annealing/extension at specific temperatures for each primer. Three samples were used for each treatment, and the experiment was repeated twice to ensure accuracy. The comparative threshold cycle method ($2^{-\Delta\Delta CT}$) was utilised for gene expression analysis, with 18s rRNA serving as the normaliser, as described by Livak and Schmittgen (2001).

2.10 | Statistical Analysis

Data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using the Bartlett's test. Following these validations, a two-way ANOVA was conducted

TABLE 2 | Gene-specific primers were used for the analysis of chicken gene expression.

Gene	Primer sequences (5'−3')	Orientation	Annealing temperature	Product size (bp)
18s	CGAAAGCATTTGCCAAGAAT GGCATCGTTTATGGTCGG	Forward Reverse	60	98
POMC	CGCTACGGCGGCTTCA TCTTGTAGGCGCTTTTGACGAT	Forward Reverse	63	88
ACC	AATGGCAGCTTTGGAGGTGT TCTGTTTGGGTGGGAGGTG	Forward Reverse	63	136
LITAF	CCCCTACCCTGTCCCACAA TGAGTACTGCGGAGGGTTCAT	Forward Reverse	63	67
HSP 70	CCAAGAACCAAGTGGCAATGAA CATACTTGCGGCCGATGAGA	Forward Reverse	60	72

Abbreviations: ACC, acetyl-CoA carboxylase; HSP 70, heat shock protein 70; LITAF, lipopolysaccharide-induced tumour necrosis is like the ALPHA factor; POMC, pro-opiomelanocortin.

to examine the effects of Factor-2 (different treated groups) within the different oil levels (Factor-1) on growth parameters, haematological parameters, and histological parameters. The analysis was performed using the general linear model (GLM) in SPSS/PC+ version 24. Post hoc comparisons were made using the Bonferroni correction test to control for type I error and false discovery rate.

3 | Results

3.1 | Growth Markers

Table 3 indicates that the final body weight was the highest in chicks fed 115% Thr with an additional 2% oil, reaching 1837.50 g, significantly outperforming other groups. This group also achieved the highest total body weight gain (1790.92 g) with significant differences noted due to the oil effect. Conversely, the lowest final body weight and weight gain were observed at the highest Thr level (145%), suggesting adverse effects of excessive Thr. Total feed intake was the highest at the lowest Thr level (100%) with control oil and the lowest at the highest Thr level with extra oil, indicating significant variances across treatments. The best FCR was seen in the 115% Thr with extra oil group, although the differences were not markedly significant. PER and EEU showed minimal significant differences, suggesting that these parameters were less affected by dietary changes. The production index was notably better at the 115% Thr and extra oil level but did not show a significant overall impact from Thr and oil levels. These data indicate that moderate increases in Thr with additional oil may optimise growth and efficiency in broiler chickens, while excessive Thr levels could be detrimental. The addition of 2% extra oil significantly improved both final body weight and total body weight gain, suggesting a positive impact on growth. This treatment also significantly affected total feed intake, a metric that was also influenced by the Thr treatment and their interaction, indicating that dietary adjustments modified feeding behaviours. However, no significant effects were observed in the FCR, PER, EEU, or PI across any treatments, implying that these dietary changes did not substantially alter metabolic efficiency or overall health and productivity. The findings high-light the growth-promoting potential of oil supplementation in bird diets while demonstrating limited impacts on metabolic and health-related parameters.

3.2 | Immune Response

The dataset from Table 4 explores the effects of varying dietary levels of Thr and oil on immune-related parameters in broiler chickens at 42 days of age. The results are presented with significance levels for the effects of Thr, oil, and their interaction on each measured parameter. Total white blood cell counts (TWBCs) significantly increased with higher levels of both Thr and oil, but the interaction between these treatments was not significant. This suggests that each treatment independently boosts TWBCs, indicative of an enhanced immune response. The percentage of lymphocytes showed no significant changes across different levels of Thr and oil, indicating stability in this aspect of immune function despite dietary changes. However, there was a significant interaction effect, suggesting subtle modulations in lymphocyte levels depending on the combined levels of Thr and oil. Heterophil percentages significantly increased with increasing levels of Thr and oil, respectively, and this parameter's increase could suggest a stress response or a shift in immune strategy, although the interaction effect was not significant. The heterophil to lymphocyte (H/L) ratio, a stress indicator, also showed significant increases in both treatments, but again the interaction was not significant. This underscores a potential stress increase as a result of higher Thr and oil levels. Phagocytic activity, reflecting the chickens' ability to fight infections, showed significant improvement with both Thr and oil treatments, without a significant interaction effect. This indicates a strengthened innate immune function.

Lastly, the phagocytic index, which measures the effectiveness of phagocytic cells, showed significant improvements with changes in the dietary regimen (for Thr and oil) with no significant interaction. Overall, the results indicate that increased levels of dietary Thr and oil enhance immune parameters such as TWBCs, heterophil percentage, and phagocytic activity, suggesting that these nutrients may play a role in boosting the immune system of

TABLE 3 | Effect of various dietary threonine and oil levels on growth performance and feed efficiency parameters of broiler chicken at 42 days of age.

	Treatment								p-value		
	Control oil				2% Extra oil						
	Thr				Thr						
Items	100%	115%	130%	145%	100%	115%	130%	145%	Thr	Oil	Interaction
Initial BW (g/bird)	48.39 ± 0.78	47.83 ± 0.69	48.10 ± 0.71	48.34 ± 0.75	48.16 ± 0.79	48.48 ± 0.85	48.31 ± 0.58	48.56 ± 0.73	.795	.714	.586
Final BW (g/bird)	1703.07 ± 61.42[ab]	1785.33 ± 48.46[ab]	1755.72 ± 62.71[ab]	1643.61 ± 49.92[b]	1741.62 ± 52.89[ab]	1837.50 ± 55.43[a]	1700.25 ± 37.97[ab]	1641.56 ± 62.91[b]	.039	.814	.673
Total BWG (g/bird)	1655.88 ± 60.78[ab]	1739.20 ± 47.96[ab]	1709.32 ± 62.19[ab]	1596.65 ± 49.36[b]	1695.12 ± 52.32[ab]	1790.92 ± 55.05[a]	1652.60 ± 37.40[ab]	1594.99 ± 62.40[b]	.036	.815	.659
Total FI (g/bird)	2914.03 ± 17.04[c]	2958.27 ± 7.88[b]	2949.88 ± 25.30[bc]	2846.54 ± 5.69[d]	3028.66 ± 9.23[a]	2824.22 ± 8.63[d]	2847.37 ± 3.70[d]	2762.87 ± 15.07[e]	.001	.001	.001
Average FCR	1.84 ± 0.09[a]	1.73 ± 0.05[ab]	1.78 ± 0.07[ab]	1.83 ± 0.06[a]	1.82 ± 0.05[a]	1.61 ± 0.04[b]	1.74 ± 0.04[ab]	1.79 ± 0.07[ab]	.059	.232	.821
Average PER	2.79 ± 0.11[b]	2.89 ± 0.08[ab]	2.85 ± 0.11[ab]	2.75 ± 0.09[b]	2.72 ± 0.09[b]	3.09 ± 0.09[a]	2.82 ± 0.06[ab]	2.80 ± 0.11[b]	.063	.533	.433
Average EEU	5.78 ± 0.28[ab]	5.45 ± 0.16[ab]	5.60 ± 0.23[ab]	5.75 ± 0.18[ab]	5.87 ± 0.17[a]	5.18 ± 0.14[b]	5.62 ± 0.13[ab]	5.76 ± 0.23[ab]	.059	.775	.807
Average PI	100.13 ± 6.72[ab]	106.65 ± 5.72[ab]	104.90 ± 7.51[ab]	94.42 ± 5.88[b]	99.67 ± 6.48[ab]	118.79 ± 7.55[a]	99.88 ± 4.45[ab]	97.74 ± 7.40[b]	.094	.582	.508

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means ± SE.

TABLE 4 | Effect of various dietary threonine and oil levels on total white blood cell counts (TWBCs), differential leucocytic counts, phagocytic activity, and index of broiler chicken at 42 days of age.

Items	Treatment								p-value		
	Control oil				2% Extra oil						
	Thr				Thr						
	100%	115%	130%	145%	100%	115%	130%	145%	Thr	Oil	Interaction
TWBCs ($\times 10^3$/mm^3) count	22.50 ± 1.33^c	32.12 ± 0.74^b	31.62 ± 1.34^b	33.12 ± 3.04^{ab}	29.62 ± 2.91^{bc}	36.75 ± 3.91^{ab}	40.50 ± 3.20^a	40.87 ± 1.54^a	.001	.001	.854
Lymphocytes (%)	37.17 ± 1.26^{ab}	38.07 ± 0.97^a	36.50 ± 0.23^{ab}	36.92 ± 0.71^{ab}	36.52 ± 0.56^{ab}	36.85 ± 1.63^{ab}	36.45 ± 1.44^{ab}	34.02 ± 1.03^b	.326	.124	.036
Heterophil (%)	42.77 ± 0.82^d	46.15 ± 0.49^c	48.67 ± 0.40^{bc}	49.22 ± 0.59^b	46.12 ± 0.64^c	47.95 ± 1.31^{bc}	49.12 ± 1.02^b	51.80 ± 1.04^a	.001	.002	.376
H/L ratio	1.15 ± 0.05^c	1.21 ± 0.04^{bc}	1.33 ± 0.02^{bc}	1.33 ± 0.04^{bc}	1.26 ± 0.03^{bc}	1.31 ± 0.09^{bc}	1.36 ± 0.08^{ab}	1.53 ± 0.08^a	.006	.020	.579
Phagocytic activity (%)	43.72 ± 0.86^a	45.70 ± 0.33^a	45.95 ± 0.60^a	45.82 ± 0.43^a	45.97 ± 0.59^a	46.52 ± 0.32^a	46.82 ± 0.36^a	46.42 ± 0.37^a	.028	.005	.381
Phagocytic index	2.80 ± 0.10^b	3.16 ± 0.08^a	3.08 ± 0.10^{ab}	3.04 ± 0.13^{ab}	3.14 ± 0.10^{ab}	3.19 ± 0.13^a	3.19 ± 0.12^a	3.21 ± 0.11^a	.047	.057	.569

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means ± SE.

broiler chickens without causing significant interaction effects. This can contribute to better health management strategies in poultry nutrition. Table 5 details the effects of varying levels of dietary Thr and oil on the relative weights of immune organs in broiler chickens at 42 days of age. For the bursa of Fabricius, no significant differences were noted due to Thr treatment, but a significant effect was observed for oil treatment, suggesting that increasing oil levels can affect the relative weight of this immune organ. The interaction between the two treatments was not significant. The thymus gland showed no significant changes in relative weight across different levels of either Thr or oil, indicating that these dietary modifications do not substantially affect the thymus gland in broilers. In contrast, the spleen showed a significant response to the Thr treatment, indicating that increasing Thr levels can influence spleen size, while oil levels and the interaction between treatments did not show significant effects. These findings suggest specific sensitivities of different immune organs to dietary components in broiler chickens, with the bursa and spleen showing variability in response to dietary changes, whereas the thymus gland remains largely unaffected.

3.3 | Blood Lipids Parameters

Table 6 presents the effects of various levels of dietary Thr and oil on blood serum lipid concentrations in broiler chickens at 42 days of age, across several lipid-related metrics. The data reveal that neither Thr nor oil treatments significantly influenced triglyceride levels, and there is no significant interaction for both Thr and oil treatments was found. Total cholesterol showed subtle variations across treatment levels but was not significantly impacted by Thr alone or oil alone; however, a significant interaction suggests that the combination of Thr and oil levels could affect total cholesterol in a more complex way.

HDL cholesterol levels also remained largely unaffected by both treatments and their interaction, with no significant changes. Similarly, VLDL levels showed stability across treatments and interactions, with no significant alterations. LDL cholesterol levels displayed no significant changes with Thr or oil individually, but there was a significant interaction effect, pointing to potential effects on LDL concentrations depending on the combination of dietary Thr and oil.

Lastly, the total cholesterol to HDL ratio (T Cho/HDL ratio) also did not show significant changes due to Thr or oil treatments independently, but a significant interaction was noted, indicating that the ratio might be influenced by how Thr and oil are combined in the diet. This table highlights that while individual components such as Thr and oil might not always independently influence lipid metrics significantly, their combined levels can interact to affect lipid profiles in broilers, particularly influencing total cholesterol, LDL, and T Cho/HDL ratios. Table 7 examines the influence of varying Thr and oil levels on liver composition in broiler chickens at 42 days. Moisture content in the liver was significantly affected by the interaction between Thr and oil treatments, indicating that the combination of these nutrients alters liver moisture. Ash content was notably influenced by oil levels and the interaction between Thr and oil, suggesting

TABLE 5 | Effect of various dietary threonine and oil levels on relative weight of immune organs of broiler chicken at 42 days of age.

	Treatment								p-value		
	Control oil				2% Extra oil						
	Thr				Thr						
Items	100%	115%	130%	145%	100%	115%	130%	145%	Thr	Oil	Interaction
Bursa	0.07 ± 0.01^b	0.07 ± 0.02^b	0.06 ± 0.02^b	0.10 ± 0.02^{ab}	0.11 ± 0.01^{ab}	0.13 ± 0.02^a	0.08 ± 0.02^b	0.08 ± 0.02^b	.333	.028	.119
Thymus gland	0.21 ± 0.04	0.19 ± 0.06	0.19 ± 0.04	0.23 ± 0.04	0.25 ± 0.02	0.27 ± 0.06	0.16 ± 0.06	0.16 ± 0.05	.570	.843	.352
Spleen	0.07 ± 0.03^b	0.16 ± 0.04^a	0.15 ± 0.02^a	0.13 ± 0.02^{ab}	0.14 ± 0.04^{ab}	0.13 ± 0.01^{ab}	0.14 ± 0.03^{ab}	0.12 ± 0.03^{ab}	.029	.835	.175

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means ± SE.

TABLE 6 | Effect of dietary threonine and oil levels on blood serum broiler lipid concentrations broiler chicken at 42 days of age.

	Treatment								p-value		
	Control oil				2% Extra oil						
	Thr				Thr						
Items	100%	115%	130%	145%	100%	115%	130%	145%	Thr	Oil	Interaction
Triglycerides (mg/dL)	203.00 ± 1.60	199.93 ± 2.72	201.48 ± 0.59	202.87 ± 1.30	196.63 ± 6.79	202.00 ± 0.50	198.95 ± 3.30	201.95 ± 1.17	.828	.413	.561
Total cholesterol (mg/dL)	196.43 ± 3.38^{ab}	198.52 ± 2.03^{ab}	204.25 ± 0.61^a	193.33 ± 7.74^{ab}	201.30 ± 2.91^a	201.88 ± 4.30^a	195.55 ± 7.95^{ab}	177.58 ± 14.59^b	.121	.366	.037
HDL (mg/dL)	54.00 ± 0.69	53.42 ± 0.50	53.25 ± 1.04	52.40 ± 1.08	54.00 ± 0.66	51.12 ± 2.30	52.40 ± 0.95	51.62 ± 1.65	.409	.277	.828
LDL (mg/dL)	101.82 ± 3.95^{ab}	105.12 ± 1.69^{ab}	110.71 ± 1.38^{ab}	100.35 ± 7.59^{ab}	107.98 ± 3.20^{ab}	110.35 ± 5.86^a	103.36 ± 9.08^{ab}	85.56 ± 13.33^b	.142	.588	.048
VLDL (mg/dL)	40.60 ± 0.32	39.98 ± 0.54	40.29 ± 0.11	40.57 ± 0.26	39.32 ± 1.35	40.40 ± 0.10	39.79 ± 0.66	40.39 ± 0.23	.828	.366	.561
T Cho/HDL ratio	3.64 ± 0.09^{ab}	3.71 ± 0.02^{ab}	3.84 ± 0.08^{ab}	3.69 ± 0.14^{ab}	3.73 ± 0.08^{ab}	3.98 ± 0.25^a	3.74 ± 0.21^{ab}	3.42 ± 0.21^b	.293	.991	.035

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means ± SE.

TABLE 7 | Effect of dietary threonine and oil levels on liver composition of broiler chicks broiler chicken at 42 days of age.

	Treatment								p-value		
	Control oil				2% Extra oil						
	Thr				Thr						
Items	100%	115%	130%	145%	100%	115%	130%	145%	Thr	Oil	Interaction
Moisture %	70.76 ± 1.58^a	72.65 ± 0.07^a	73.36 ± 1.85^a	74.79 ± 0.30^a	74.45 ± 0.09^a	71.87 ± 0.03^a	72.20 ± 2.45^a	65.49 ± 2.87^b	.347	.113	.007
Ash %	1.36 ± 0.12^{bc}	1.22 ± 0.02^c	1.30 ± 0.06^c	1.39 ± 0.02^{bc}	1.35 ± 0.01^{bc}	1.58 ± 0.08^a	1.49 ± 0.01^{ab}	1.59 ± 0.04^a	.172	.001	.039
CP %	14.84 ± 0.69	15.71 ± 1.44	14.84 ± 1.34	14.28 ± 1.85	15.87 ± 0.98	16.95 ± 0.46	18.03 ± 2.16	15.35 ± 0.27	.556	.096	.811
Fat %	5.57 ± 0.18^{bc}	6.29 ± 0.02^b	4.05 ± 0.20^c	5.78 ± 0.19^{bc}	4.96 ± 0.08^{bc}	5.36 ± 0.14^{bc}	5.96 ± 0.60^b	13.26 ± 1.45^a	.001	.001	.001

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means $\pm$ SE.

that oil significantly impacts the mineral composition of the liver. The crude protein percentage remained stable across all treatments, showing no significant changes due to dietary levels of Thr and oil. However, fat content in the liver was significantly altered by both Thr and oil levels and their interaction, indicating a profound influence on liver fat composition. These results highlight that while protein content is stable, moisture, ash, and fat in the liver are sensitive to changes in dietary Thr and oil levels.

3.4 | Intestinal Morphology and Absorptive Capacity

The results in Table 8 and Figures 1–4 reveal that the higher dietary Thr with different oil levels significantly (p ≤ .05) increased the villus height. The best intestinal morphology and absorptive capacity (greatly improved villus height and width of jejunum and ileum) were observed at 115% Thr with high dietary oil. Thr supplementation with low or high nutritional oil content increased the goblet cell number (GN) in the jejunum and ileum. This increase in the GN was significant at 115% Thr and non-significantly with the higher dietary Thr levels (130% and 145%).

3.5 | Expression of Genes Related to Feed Intake, Immunity, and Lipid Metabolism

Thr supplementation with low dietary oil content showed a non-significant effect on the expression of POMC. The gene was slightly up-regulated in the control group with high nutritional oil to 1.7-fold. Meanwhile, the higher Thr level (145%) significantly increased the gene expression to 9.8-fold. However, the combinations of high oil and 115% and 130% Thr are associated with slight down-regulation of the gene, as shown in Figure 5A. Regarding ACC gene expression, Thr supplementation with low dietary oil showed mild up-regulation in the gene expression to 4.9-, 1.7-, and 2.75-fold with 115%, 130%, and 145% Thr levels, respectively. With high oil, ACC gene expression was up-regulated to 5.7-fold in the control group, threefold with Thr 130%, and a significant level (16.4) with Thr 145%. The combination of high oil and 115% Thr is associated with slight down-regulation of the gene expression, as Figure 5B reveals.

The present data in Figure 5C reveal that different Thr levels with low oil showed an invariant effect on HSP70 gene expression linked to broiler chick groups fed basal diet. With high oil, marked up-regulation of the gene expression was observed in the basal diet group, and the groups received 130% and 145% Thr to 14.5-, 7.2-, and 26.5-fold, respectively. However, with 115% Thr, the lowest expression of the gene (2.27-fold) was observed. Regarding LITAF gene expression, low and high oils with different Thr levels showed variable gene expression levels. With low crude, the up-regulation of the gene was observed to 3.4- and 9-fold with 115% and 130% Thr, respectively, and down-regulation to 0.6-fold with 130% Thr. Also, extra oil addiction with Thr levels at 100%, 130%, and 145% unregulated the expression of the gene to 13-, 5.7-, and 62.9-fold, respectively; meanwhile, the higher dietary oil with 115% Thr caused mild up-regulation to threefold expression as shown in Figure 5D.

TABLE 8 | Effect of various dietary threonine and oil levels on morphology, absorptive capacity, and goblet cell density of jejunum and ileum of broiler chicken at 42 days of age.

Items	Control oil				2% Extra oil				p-value		
	Thr				Thr				Thr	Oil	Interaction
	100%	115%	130%	145%	100%	115%	130%	145%			
Jejunum											
Villi height (VH) (μm)	1191.6 ± 34.13^{cd}	1597.2 ± 74.00^{b}	1238.7 ± 54.82^{cd}	1761.2 ± 86.57^{b}	1208.4 ± 65.92^{cd}	1964.9 ± 55.57^{a}	1036.0 ± 107.2^{d}	1294.5 ± 25.13^{c}	.001	.001	.001
Villi width (VW) (μm)	337.2 ± 13.96^{a}	341.6 ± 22.379^{a}	283.8 ± 39.58^{ab}	228.4 ± 15.75^{b}	268.2 ± 26.24^{ab}	301.5 ± 41.71^{ab}	294.4 ± 35.59^{ab}	218.4 ± 21.11^{b}	.020	.202	.550
Crypt depth (CD) (μm)	340.9 ± 31.33^{a}	248.5 ± 23.59^{ab}	235.1 ± 6.63^{b}	242.2 ± 37.49^{ab}	254.5 ± 12.63^{ab}	227.98 ± 19.41^{b}	276.5 ± 39.13^{ab}	229.2 ± 51.11^{b}	.025	.383	.271
Villi: Crypt ratio	3.55 ± 0.31^{de}	6.59 ± 0.85^{abc}	5.29 ± 0.39^{bcd}	7.73 ± 1.54^{ab}	1.36 ± 0.18^{e}	8.79 ± 1.02^{a}	3.90 ± 0.63^{cde}	$6.19 \pm 0. 1.21^{abcd}$	.001	.262	.096
Goblet cell density (no ×10³/mm²)	1.48 ± 0.07^{b}	2.07 ± 0.10^{a}	1.54 ± 0.15^{b}	1.86 ± 0.05^{ab}	1.51 ± 0.06^{b}	2.42 ± 0.12^{a}	1.51 ± 0.02^{b}	1.67 ± 0.06^{b}	.022	.518	.037
Ileum											
Villi height (VH) (μm)	764.2 ± 30.0^{d}	1223.6 ± 12.7^{bc}	1151.4 ± 80.9^{bc}	1361.8 ± 112.2^{b}	832.9 ± 7.37^{d}	1685.9 ± 76.7^{a}	806.3 ± 30.9^{d}	1096.8 ± 129.4^{c}	.001	.710	.001
Villi width (VW) (μm)	435.7 ± 21.4^{c}	461.1 ± 4.69^{c}	496.5 ± 26.6^{c}	475.1 ± 31.8^{c}	436.0 ± 14.7^{c}	621.8 ± 50.8^{b}	435.7 ± 21.3^{c}	713.2 ± 24.4^{a}	.001	.001	.001
Crypt depth (CD) (μm)	181.9 ± 5.36^{e}	663.9 ± 62.8^{a}	402.7 ± 41.3^{cd}	381.0 ± 20.7^{d}	193.9 ± 6.68^{e}	495.2 ± 51.6^{bc}	181.8 ± 5.58^{e}	548.8 ± 23.3^{b}	.001	.046	.001
Villi: Crypt ratio	4.19 ± 0.06^{ab}	1.87 ± 0.14^{d}	2.94 ± 0.46^{c}	3.62 ± 0.48^{abc}	4.30 ± 0.12^{ab}	3.47 ± 0.37^{bc}	4.44 ± 0.21^{a}	1.99 ± 0.15^{d}	.001	.078	.001
Goblet cell density (no ×10³/mm²)	1.84 ± 0.06^{b}	2.76 ± 0.08^{a}	1.86 ± 0.07^{b}	2.16 ± 0.12^{b}	1.96 ± 0.07^{b}	2.99 ± 0.11^{a}	1.94 ± 0.08^{b}	2.03 ± 0.11^{b}	.013	.981	.004

Note: Means denoted within the same row with different superscripts are significant ($p < .05$). Values are expressed as means ± SE.
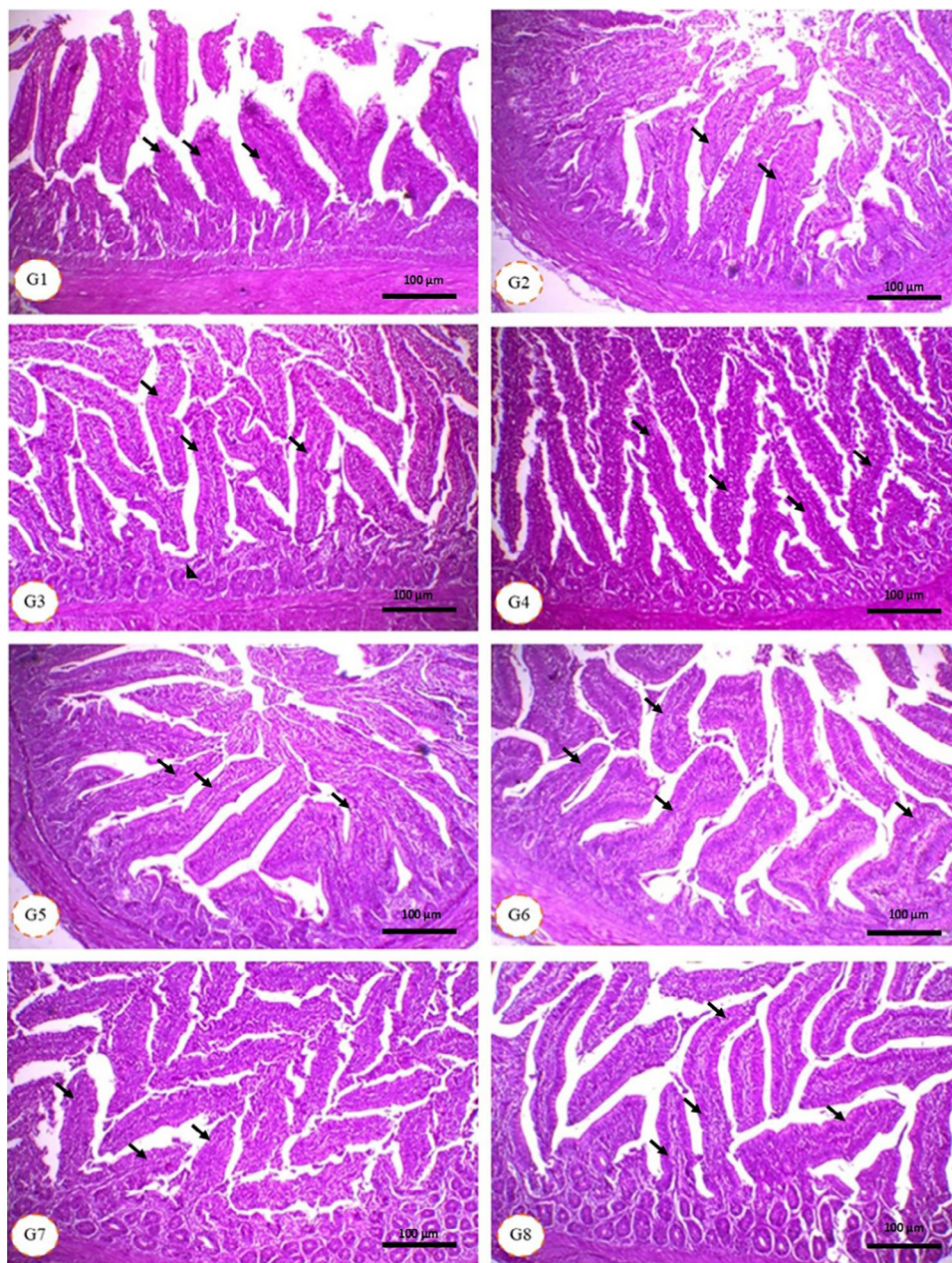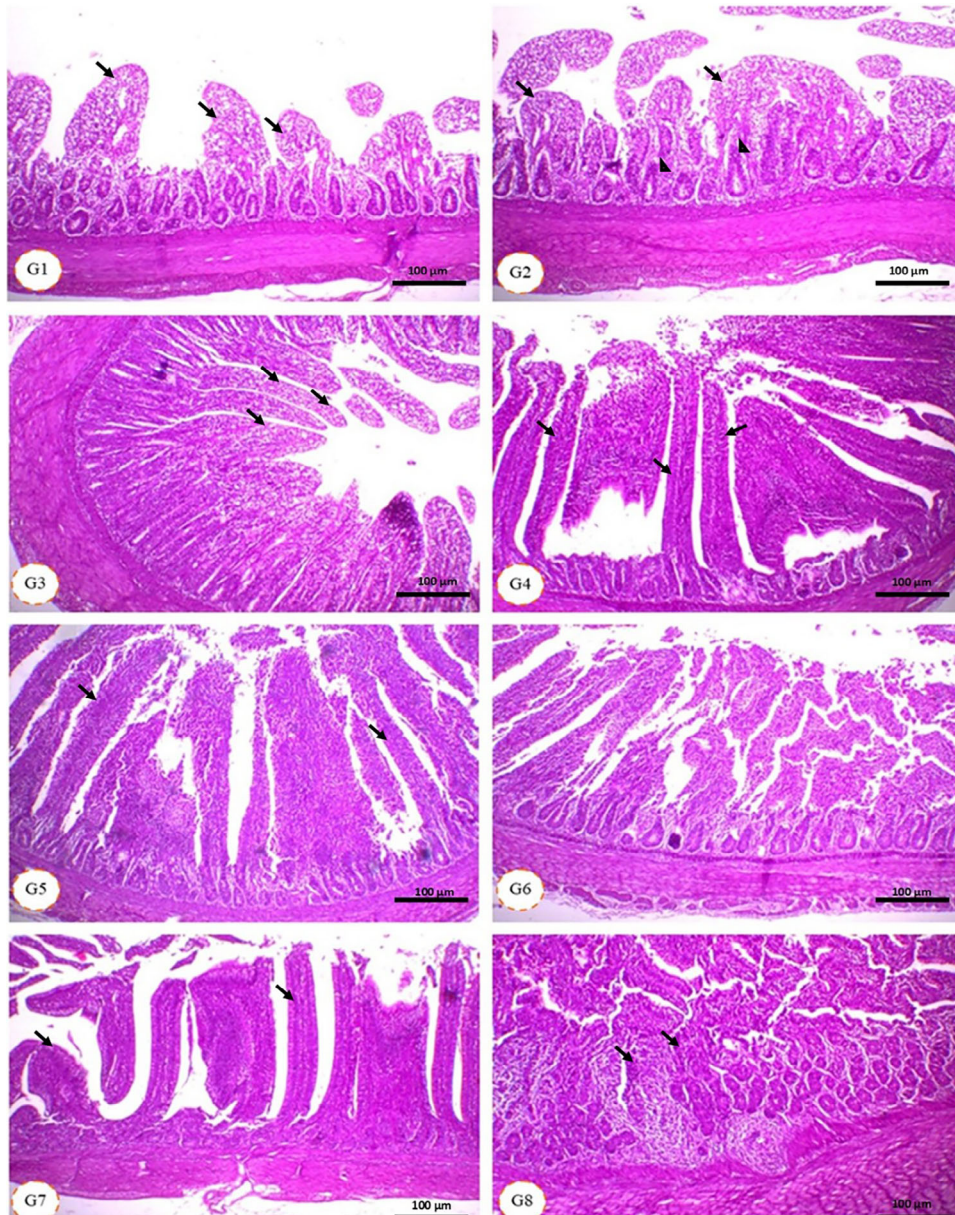
FIGURE 1 | Effect of various dietary threonine and oil levels on morphology and absorptive capacity of the jejunum of broiler chickens. G1 Thr level 100% from a recommended catalogue with low oil. The jejunum of G1 shows normal intestinal villi lined with pseudostratified epithelium (black arrow). G2 = Thr level 100% from the recommended catalogue with high oil; jejunum of G2 shows a slight increase in intestinal villi length and thickness (black arrow). G3 = Thr level 115% from a recommended catalogue with low oil; jejunum of G3 showed highly branched uniformly distributed and elongated intestinal villi (black arrow), with visible crypts of Lieberkühn (arrowhead). G4 = Thr level 115% from a recommended catalogue with high oil; jejunum of G4 shows an increase in the numbers and branches of well-preserved intestinal villi (black arrow). G5 = Thr level 130% from a recommended catalogue with low oil; jejunum of G5 shows a moderate increase in intestinal villi width and length (black arrow). G6 = Thr level 130% from a recommended catalogue with high oil; jejunum of G6 shows a slight increase in intestinal villi width and length (black arrow). G7 = Thr level 145% from a recommended catalogue with low oil; jejunum of G7 shows a marked increase in intestinal villi (black arrow). G8 = Thr level 145% from a recommended catalogue with high oil. The jejunum of G8 shows a marked increase in villi length, width, and branches, with normal intestinal villi lined with normal pseudostratified epithelium (black arrow).

4 | Discussion

The broiler chicken has higher essential AA requirements due to its fast growth rate and its consumption of less feed per unit of weight gain (Dozier et al. 2010). It was recorded that the best growth performance and feed efficiency parameters were observed with increasing dietary Thr level to 115% combined with high oil, and there was a linear body weight reduction with the increase in Thr levels of more than 115. No variation in final body weight and weight gain was observed with low dietary oil; the same result was obtained by Htin et al. (2007), who described that the body weight of chicks did not differ substantially with
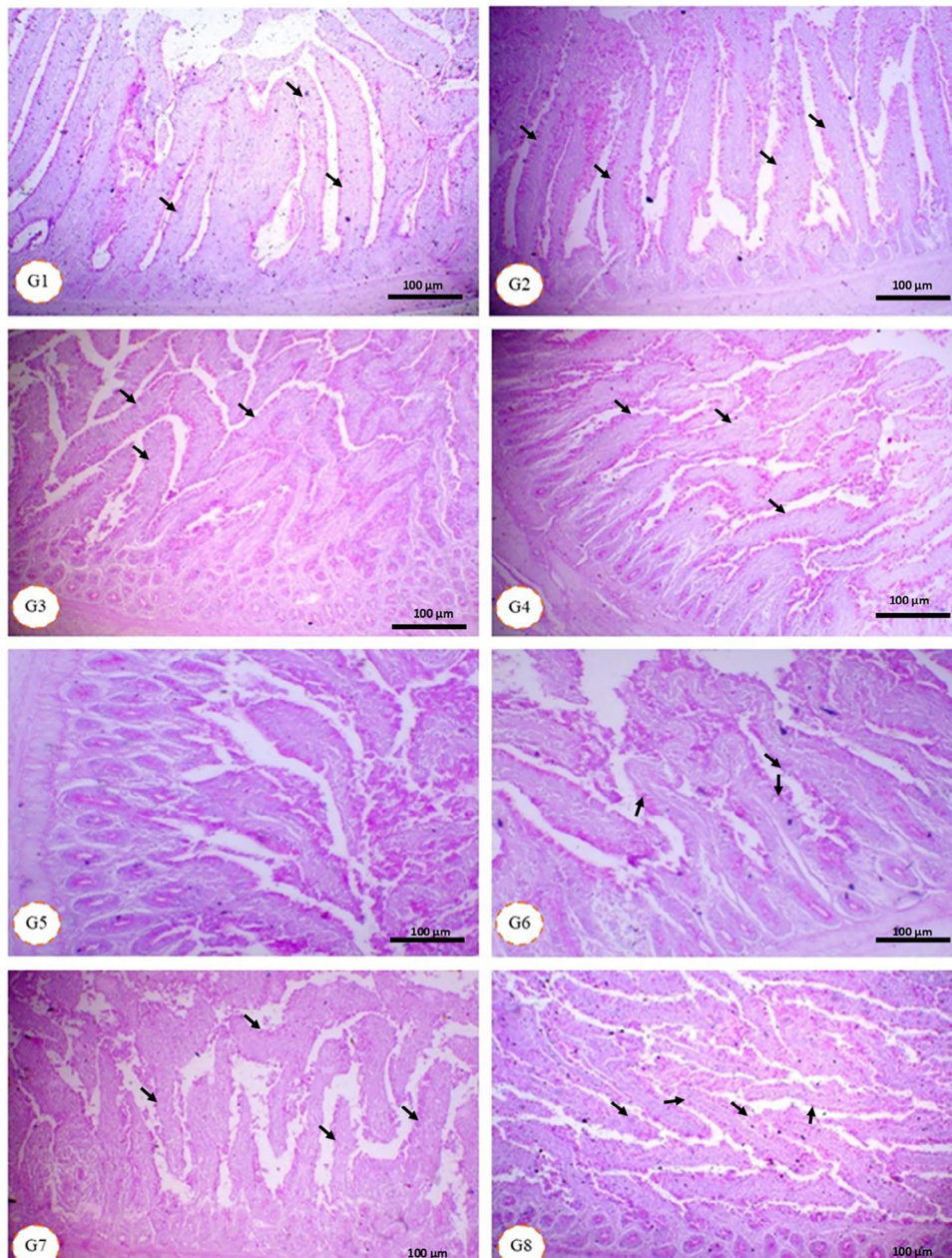
FIGURE 2 | Effect of various dietary threonine and oil levels on morphology and absorptive capacity of ileum of broiler chickens. G1 = Thr level 100% from a recommended catalogue with low oil; ilium of G1 shows normal intestinal villi and crypt (black arrow). G2 = Thr level 100% from a recommended catalogue with high oil; ilium of G2 showing an increase in the uniformly distributed intestinal villi width (black arrow) and depth of the crypts (arrowhead). G3 = Thr level 115% from a recommended catalogue with low oil. ilium of G3 offers marked intestinal villi length (black arrow) and crypt depth. G4 = Thr level 115% from a recommended catalogue with high oil; ilium of G4 shows an apparent increase of intestinal villi length and width (black arrow). G5 = Thr level 130% from a recommended catalogue with low oil; ilium of G5 showing moderate intestinal villi length and width (black arrow). G6 = Thr level 130% from a recommended catalogue with high oil; ilium of G6 showing moderate intestinal villi length and width (black arrow). There is noticeable cellular infiltration in the mucosal layer. G7 = Thr level 145% from a recommended catalogue with low oil; ilium of G7 showing moderate intestinal villi length and width. G8 = Thr level 145% from a recommended catalogue with high oil showing a marked increase in villi length and width.

palm oil, soybean oil, fish oil, and corn oil added rations at day 35.

AAs regulate specific and non-specific immunity during immune challenges (Li et al. 2007). Our data indicate that higher dietary levels of Thr, in conjunction with varying oil levels, led to an increase in lymphocytes, heterophile%, phagocytic activity, and phagocytic index, suggesting an enhancement of the immune response. These findings are supported by Corzo et al. (2007), who reported a similar increase in heterophil and lymphocyte percentages with higher dietary levels of Thr while observing no effect on the H/L ratio in Ross × Ross male chicks. Additionally, Moghaddam and Emadi (2014) noted a non-significant impact on lymphocytes, heterophile%, and the H/L ratio with higher dietary Thr levels. Similarly, Mahmoud, Ibrahim, and Badawi (2013) concluded that the higher inclusion of olive oil led to
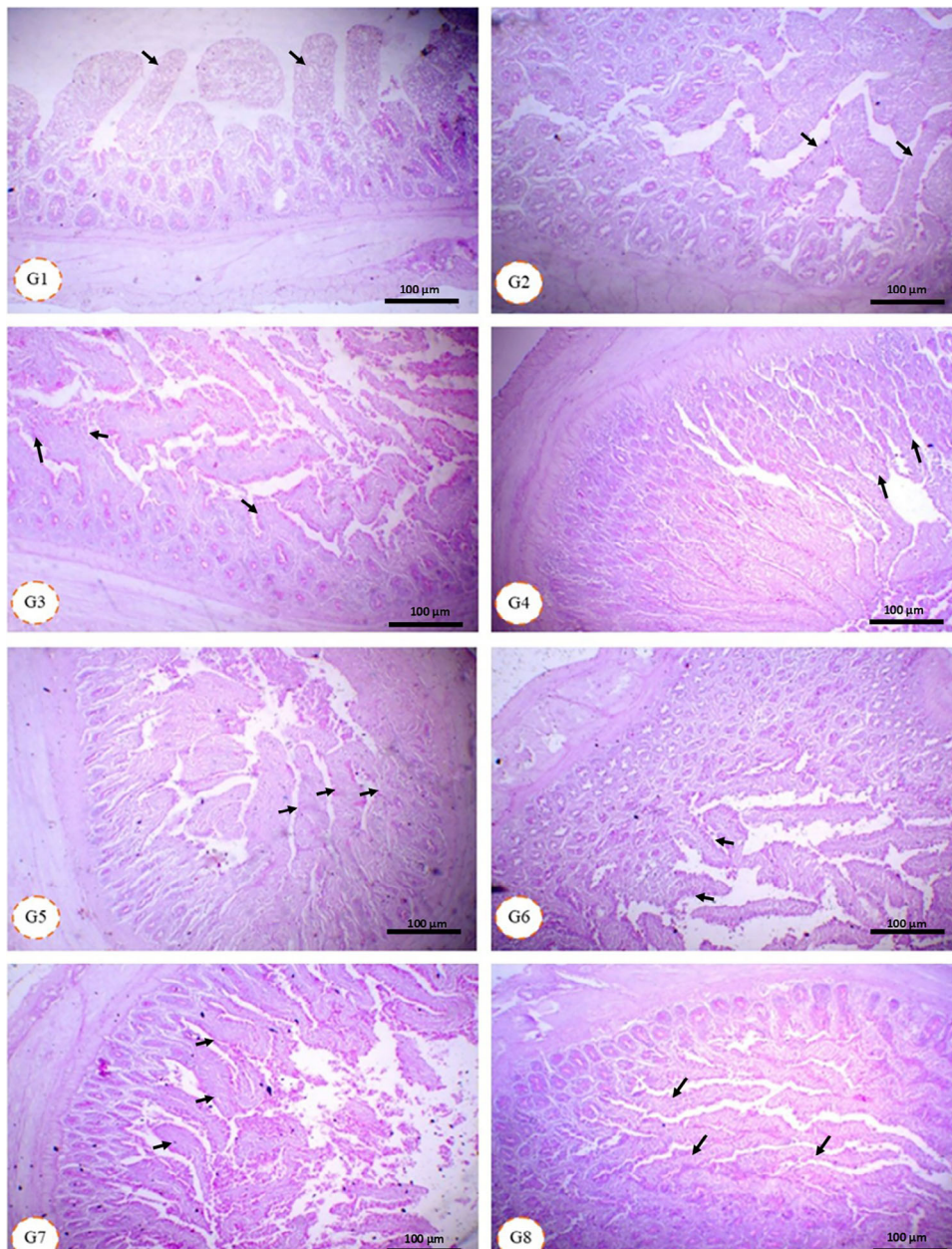
FIGURE 3 | Effect of various dietary threonine and oil levels on broiler chickens' goblet cell number of jejunum. G1 = Thr level 100% from a recommended catalogue with low oil; jejunum of G1 shows normal intestinal villi and magenta-stained goblet cells within the lining epithelium of intestinal (black arrow)—PAS, villi. G2 = Thr level 100% from a recommended catalogue with high oil; jejunum of G2 shows a moderate increase in intestinal villi length and thickness and goblet cells (black arrow)—PAS. G3 = Thr level 115% from a recommended catalogue with low oil—PAS; jejunum of G3 marked a moderate branched intestinal villi increase and goblet cells (black arrow). PAS, G4 = Thr level 115% from a recommended catalogue with high oil; jejunum of G4 shows an average increase in the number and branches of intestinal villi and goblet cells (black arrow). PAS, G5 = Thr level 130% from a recommended catalogue with low oil; jejunum of G5 shows a moderate increase in intestinal villi width and length and in goblet cells (black arrow). PAS, G6 = Thr level 130% from a recommended catalogue with high oil; jejunum of G6 showing a mild rise in intestinal villi width and length and in the number of goblet cells (black arrow). PAS, G7 = Thr level 145% from a recommended catalogue with low oil; jejunum of G7 showing a moderate increase in intestinal villi and goblet cells (black arrow). PAS, G8 = Thr level 145% from a recommended catalogue with high oil. Jejunum of G8 shows a marked increase in villi length, width, and branches and the number of goblet cells (black arrow).

a non-significant increase in heterophile and monocyte count and had a non-significant effect on lymphocytes. Threonine's role in enhancing the synthesis of tight junction proteins or modulating cellular turnover could be elaborated (Yang and Liao 2019). Additionally, integrating comparative studies similar to those by Johnson et al. (2021), which documented variations in immune response due to different AA levels, would provide a broader scientific context. Such a comprehensive approach ensures the discussion not only delineates how Thr contributes to physiological functions but also situates these findings within the larger framework of nutritional science, encouraging further exploration into Thr's multifaceted roles (Zhang and Kim 2014).
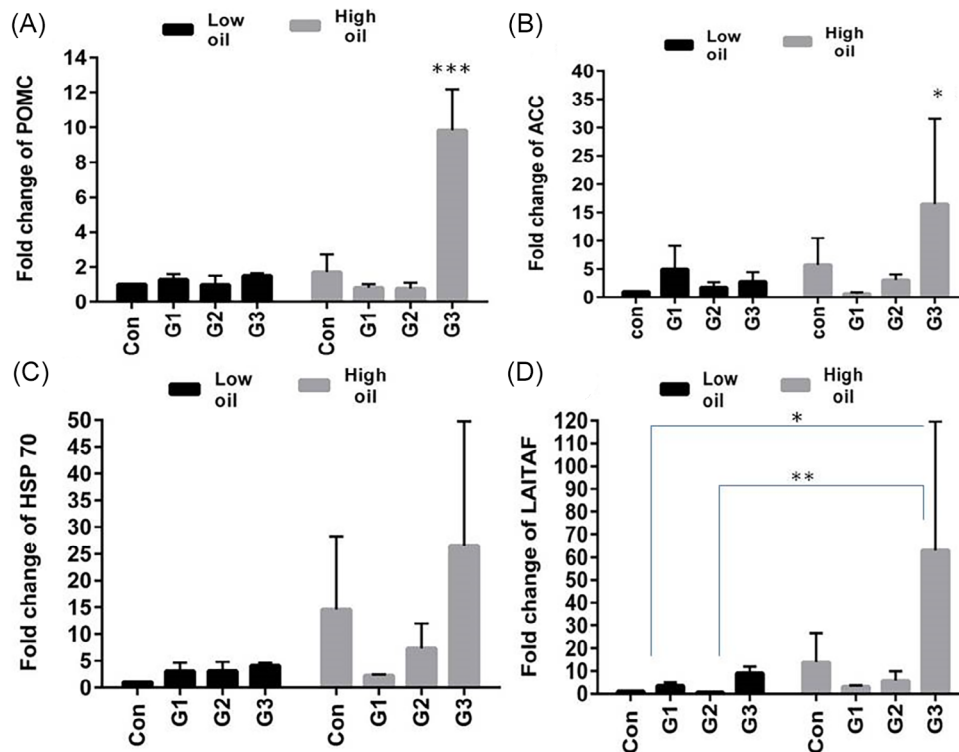
FIGURE 4 | Effect of various dietary threonine and oil levels on goblet cell number of ileum of broiler chickens. G1 Thr level 100% from a recommended catalogue with low oil; ilium of G1 shows normal intestinal villi and goblet cells within the crypt of Luberkuhn (black arrow). PAS, G2 = Thr level 100% from a recommended catalogue with high oil; ilium of G2 shows a moderate increase of intestinal villi and the goblet cell number. PAS, G3 = Thr level 115% from a recommended catalogue with low oil; ilium of G3 shows highly branched intestinal villi and a marked rise in the goblet cell number (black arrow). PAS, G4 = Thr level 115% from a recommended catalogue with high oil; ilium of G4 shows a moderate rise in the number and branches of intestinal villi and the goblet cell number (black arrow). PAS, G5 = Thr level 130% from a recommended catalogue with low oil; ilium of G5 shows slight to moderate increase in intestinal villi width and length and the goblet cell number (black arrow). PAS, G6 = Thr level 130% from a recommended catalogue with high oil; ilium of G6 shows a mild rise in intestinal villi width and length the goblet cell number (black arrow). PAS, G7 = Thr level 145% from a recommended catalogue with low oil; ilium of G7 shows a marked increase in intestinal villi. The villi are elongated shape and increased goblet cell number (black arrow). PAS, G8 = Thr level 145% from a recommended catalogue with high oil; ilium of G8 shows most pronounced villi development and a rise in the goblet cell number (black arrow).

Effects of the combination of different dietary oils and Thr level in our lymphoid organ weight study. Thr supplementation with low oil improved spleen weight, while 115% Thr with high oil increased bursa weight. Kwak, Austic, and Dietert (1999) graded dietary levels of arginine AA affect lymphoid organ weight, with an apparent impact on the spleen and thymus than the bursa of Fabricius. Except for the observed lower serum cholesterol and LDL levels by combining the high oil and 145% Thr, no changes were followed in the serum lipid profile by the other combinations of oil and Thr levels. Several authors have also reported that the different dietary oil levels did not affect the serum triglycerides, cholesterol, and HDL concentrations

FIGURE 5 | Effect of various dietary threonine and oil levels on the expression of genes related to feed intake, immunity, and lipid metabolism of broiler chicks.(A) POMC, pro-opiomelanocortin; (B) ACC, acetyl-CoA carboxylase; (C) HSP70, heat shock protein 70; (D) LITAF, lipopolysaccharide-induced tumour necrosis like alpha factor Con=basal diet with 100% Thr, G1: 115% Thr, G2: 130% Thr, G3: 145% Thr. *Significant at $p \leq .01$; **significant at $p \leq .001$; ***significant at $p \leq .000$.

(Rezaeipour, Alinejad, and Asadzadeh 2016). However, Alparslan and Özdogan (2006) found that higher dietary fish oil inclusion significantly lowered serum triglyceride and cholesterol levels.

One target of broiler ration is to avoid the fatty life; high fat imbalanced rations are usually associated with fatty liver because of extreme production of fatty acids from carbohydrates, depressed metabolism, and oxidation and/or reduced transport of fat from the liver (Searcey and Arata 1972). In our data, the combination of high oil and Thr (145%) caused decreases in moisture %, associated with a significant increase in liver fat %. However, the best combination showed a substantial reduction of liver lipids, with the group receiving high oil and 115% Thr. Parallel with our result, Ogura and Nakamura (1963) observed that 0.2% Thr supplementation increased protein utilisation with a lowered fat accumulation in the rat's liver. Additionally, Shin, Kakani, and Karimi (2011) reported that high flaxseed and fish oil ration showed a non-significant effect on the liver fat content and explained that higher dietary omega-3 content prevented the fatty liver.

Our study's overall assessment of morphology, absorptive capacity, and goblet cell density of jejunum and ileum indicates an improvement of almost all criteria in the group that received the combination of high oil and 115% Thr. This group also showed better feed efficiency, the best growth performance, and average FCR. The surface area for nutritional absorption is enhanced by an increase in crypt depth, achieved through a rise in enterocyte proliferation and mucin secretion (Tsirtsikos et al. 2012). Also, Soltan (2009) demonstrated increased intestinal surface area

and nutrient absorption associated with increased villus height and better bird performance. These results are supported by de Barros Moreira Filho et al. (2015), who observed that dietary L-threonine AA improved the intestinal morphology of the broilers. Furthermore, Min et al. (2017) stated that supplementation with Thr led to a linear or quadratic increase in villi height, depth, and VH/CD ratio. The high-fat diet enhances the villus growth and up-regulated the expression of CD36, which may play a role in post-resection high-fat diet-induced villus growth (Choi et al. 2014).

The impact of Thr on intestinal development and mucosal production, demonstrating that 60% of Thr in the broiler ration is maintained by the intestine, with 80% utilised by enterocytes (Horn et al. 2009). They have also shown that dietary Thr can impact mucin secretion by goblet cells and influence intestinal mucin dynamics. Thus, a consistent supply of Thr is crucial for gut function and structure (Law et al. 2007). Additionally, de Barros Moreira Filho et al. (2015) described positive outcomes from Thr supplementation on the goblet cell count in the jejunum and ileum. In our study, a dietary Thr level of 115% and two different oil levels substantially improved goblet cell numbers in the jejunum and ileum compared to the control and other Thr levels (130% or 145%). This finding may be explained by X. Wang et al. (2007), who examined a decline in the synthesis rate of fractional protein in the small intestine associated with an imbalance in dietary Thr intake, whether deficient or in excess. An imbalance in dietary Thr, whether deficient or excessive, can alter the synthesis rates of fractional proteins in the small intestine. Such disruptions can impair the functionality of goblet

cells, which are crucial for mucin production and maintaining intestinal barrier integrity. Thus, the optimal 115% Thr level likely supports appropriate protein synthesis, enabling optimal goblet cell function and enhancing mucosal protection. In contrast, higher levels (130% and 145%) may disrupt this balance, leading to a decline in goblet cell effectiveness. This dynamic highlights the importance of maintaining balanced dietary Thr levels for optimal intestinal health.

Hypothalamic appetite-related peptides are primarily affected by dietary fat (X. J. Wang et al. 2017). Regarding the gene expression data of POMC, the gene expression level detected for each group directly reflects the total feed intake data presented in Table 4. Although Thr supplementation with low dietary oil content showed a non-significant effect on the expression of POMC, there was slight up-regulation in the group that received low oil, and 145 % Thr showed lower feed intake compared with the control and other levels of Thr. Additionally, with high oil, the most negligible feed intake was observed with Thr level (145%), significantly increasing POMC gene expression to 9.8-fold. Our results match with those of Liu et al. (2016), who proposed that the high-fat diet led to an increase in the expression of POMC gene expression.

The primary goals of the poultry industry are to use a different dietary composition to regulate lipid metabolism, reduce abdominal fat content, and increase carcass yield. ACC is valuable in fatty acid synthesis and degradation (Rosebrough, Russell, and Richards 2011). In mice, the activation of ACC enzymes was associated with increased accumulation of hepatic fat (Fullerton et al., 2013). Different Thr supplementations with low dietary oil showed mild up-regulation of ACC gene expression. With high oil, the lowest expression level of ACC was observed in the group that received 115% Thr (0.6-fold), while the highest expression was observed in the group that received Thr 145% (16.4-fold). Also, this group has significantly higher liver fat, lower total cholesterol, and a higher cholesterol/HDL ratio than other groups. A fat deposition usually needs an increased energy supply, resulting in energy loss and reduced feed efficiency (Duarte et al. 2014). Furthermore, the group with high oil and 145 % Thr also has the highest ACC and POMC gene expression, reflected by the increased fatty liver, lowest feed intake, and weight gain. Our results are in linearity with other reports that suggested that the ACC gene expression stimulates the malanonyl-CoA and long-chain fatty acyl-CoA as indicators of the status of hypothalamus energy and increases the expression of the hypothalamic anorexigenic gene (POMC) (Carling, Sanders, and Woods 2008; Lage et al. 2008). Heat shock proteins (HSP) are protecting proteins used as biomarkers of various stresses (Horowitz 2007). In our study, the rearing of the birds was from 11 May until 22 June, when the ambient temperature ranged from 30 to 33, which exceeded the optimum temperature range for chickens and can be considered chronic heat stress (Lin et al. 2004). Regarding the expression of HSP70, mild up-regulation of the gene was detected with low oil and different Thr levels. Meanwhile, high oil with a higher expression level was observed in the group that received a basal diet of Thr 130% and 145%. The slightest expression of Hsp70 was observed in the group that received high oil and 115% Thr. Gabriel et al. (2000) found that dietary energy greatly influenced the hepatic Hsp70 expression, and the thermo-resistance of the broiler during heat stress could

be changed by feeding a high-energy diet as the Hsp70 level was meaningfully decreased in high-energy fed birds—alteration of inflammatory cytokines mRNA expression observed with different oil sources in broiler chickens (Ibrahim et al. 2018). In our study, other dietary oils and Thr showed variable effects on the gene expression of LITAF genes. With low oil, the least expression was observed with 130% Thr; meanwhile, its level increased to 3.4- and 9-fold with 115% and 145%, respectively. With high oil, the basal diet without Thr showed a 13-fold increase in the gene expression, the least expression with 115% Thr, and the highest was observed with 145 % Thr (26.9), indicating the role of Thr level in altering the expression of LITAF gene. Bhanja et al. (2015) observed the variable stimulatory role of Thr and Cyt AAs on the expression of inflammatory and immune gene expression in ovo AA administration.

5 | Conclusion

The combination of high oil and 115% Thr levels has been identified as optimal for broiler health and productivity, leading to enhanced growth performance, improved immune parameters, and better intestinal health. This dietary regimen correlates with decreased expression of key genes such as ACC, POMC, HSP70, and LITAF, which in turn results in a superior FCR, reduced fatty liver occurrence, and improved thermo-resistance and immune status in the birds. A practical takeaway from these findings is that poultry diets formulated to include 115% of recommended Thr levels and higher oil content can be strategically used to boost overall health and efficiency in broiler production. This approach not only supports the well-being of the birds but also enhances the economic viability of poultry farming by improving key productivity metrics.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed. The study was authorised by the

institutional ethics committee at Alexandria University in Egypt and conducted following the guidelines established by the local experimental animal care committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are available upon request from the corresponding authors.

Peer Review

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