SUPPLEMENTATION OF AQUAGEST® AS A SOURCE OF MEDIUM-CHAIN FATTY ACIDS AND TAURINE IMPROVED THE GROWTH PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, AND IMMUNE RESPONSE OF COMMON CARP (CYPRINUS CARPIO) FED LOW FISH MEAL DIETS

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Abstract

Four diets were prepared to include a mixture of medium-chain fatty acids and taurine as a digestive/metabolic enhancer (DME, AQUAGEST®) at 0, 1, 2, and 3 g DME/kg diet and fed to common carp (initial weight, 4.55±0.03 g) for 70 days. Dietary DME significantly increased the final weight, weight gain, specific growth rate, feed intake, and protein efficiency and decreased feed conversion ratio in a dose-dependent manner (P<0.05). The body lipid composition was significantly improved by feeding DME at 2 g/kg diet (P=0.0141). The intestine villus length and the number of goblet cells were significantly increased in fish fed 2 g DME/kg diet (P<0.05). The intestinal villi displayed increased length, branching, and density by supplementing DME to common carp diets. Fish fed DME at 2 g/kg diet displayed markedly decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (P=0.025 and P=0.043) and increased total protein and globulin (P =0.002 and P=0.003). Additionally, fish fed 2 and 3 g DME/kg levels displayed significantly increased albumin levels (P=0.006). Lysozyme and phagocytotic activities were increased by feeding DME at 2 g/kg diet, while the phagocytic index increased by 2 and 3 g/kg diet (P<0.05). The optimal supplementation level of DME is 1.63 to 2.05 g/kg for common carp based on the polynomial regression analysis. In conclusion, common carp fed diets with a mixture of medium-chain fatty acids and taurine displayed improved growth, digestion activity, and immune response.

Key words: medium-chain fatty acids, taurine, common carp, growth performance, intestinal histomorphology, immune response
In aquaculture, unsuitable farming conditions can affect the feeding habits of fish and, in turn, impair the health condition (El Megid et al., 2020). For instance, in intensive aquaculture systems, the amounts of natural feed sources decrease, and nutritionally balanced diets are required to maintain the healthy growth of fish (Gaber et al., 2012; Sithara and Kamalaveni, 2008). Common carp (Cyprinus carpio) is a freshwater fish that is easy to culture, resistant to disease, can live in tropical climates, and has high economic value (Sarhadi et al., 2020). Common carp are classified as herbivorous fish species based on their feeding habits that can accept high levels of carbohydrates with relatively low levels of protein when compared with carnivorous fish species (Webster and Lim, 2001). However, intensive rearing conditions impair the growth performance and health condition of common carp via reducing the feed intake (Dawood et al., 2019a, Dawood et al., 2020d). The coefficient digestibility rate shows low levels of digested carbohydrates due to the high levels of non-digested fibers and inappropriate rearing conditions (Dossou et al., 2018).

Dietary medium-chain fatty acids (MCFA) and taurine are usually used in aquafeeds as growth promoters and metabolic enhancers (Tran et al., 2018; Abdel-Tawwab, 2016). The MCFA is produced via the fermentation of carbohydrates with anaerobic bacteria in the gastrointestinal tract of the organism (Bedford and Gong, 2018). The produced MCFA mainly potentiates the beneficial microbiota to digest and absorb the nutrients through the intestinal villi and indirectly alleviate the activity of pathogenic bacteria (Hoseinifar et al., 2017). Therefore, the inclusion of MCFA can result in markedly enhanced growth performance, feed efficiency, antioxidant activity, and immune responses (Zhou et al., 2019; Mirghaed et al., 2019; Dawood et al., 2020a).

Taurine, on the other hand, is an essential free amino acid that is present in high amounts in animal protein sources (e.g., fish meal) (Li et al., 2016; Abdel-Tawwab and Monier, 2018). Taurine can be synthesized from methionine via cysteine metabolism, which is varied among fish species due to the availability of sulfur-containing amino acids and the difference of feeding habits (Kim et al., 2008; Gibson Gaylord et al., 2007). Taurine synthesis ability is species-specific; thus, taurine requirements are varied among fish species (Dong et al., 2018; Yan et al., 2019). Taurine is required for supplying essential metabolites and synthetic reactions, osmotic regulation, detoxification processes, and nervous system function (Shen et al., 2019; Stipanuk et al., 2002). Also, taurine has antibacterial, immunomodulatory activity, and antioxidant properties in aquatic animals (Zhang et al., 2018; Espe et al., 2012; Hoseini et al., 2018). Besides, taurine has been reported to regulate and help in the metabolism of lipids in the fish body (Hoseini et al., 2018; Kim et al., 2008). The plant ingredients have a low amount of taurine; therefore, when preparing plant protein-based diets, taurine must be added at reasonable levels (Hoseini et al., 2017; Hoseini et al., 2018).

Due to the deficiency of fish meal inclusion in the diets of herbivorous species, including common carp, it is then crucial to include taurine in fish diets for the optimal growth performance and wellbeing (Abdel-Tawwab and Monier, 2018). The combination of MCFA and taurine is proposed to present beneficial effects for aquatic animals. Hence, the present study was conducted to investigate the potential role of MCFA and taurine mixture as a digestive/metabolic enhancer on the growth performance, intestinal histomorphology, and health condition of common carp.
Material and methods

Diet preparation

Four test diets were prepared with the inclusion of a digestive/metabolic enhancer (DME) (a mixture of medium-chain fatty acids (MCFA) and taurine at the rate of (2.3:4) [AQUAGEST® OMF, Nutri-Ad International NV, Belgium]). The DME was included at 0, 1, 2, and 3 g/kg diet in the presence of wheat flour as a filler. The basal diet was formulated by the inclusion of fish meal at a low level (2%), soybean meal (21%), and poultry by-product meal (20%) as protein sources. The gluten, wheat bran, rice bran, yellow corn, and flour were used as carbohydrate sources and binders. All ingredients were well mixed in the presence of water, oil, dicalcium phosphate, and vitamin and minerals mixture; then, the dough was subsequently extruded by a pelleting machine through a die of 1 to 2 mm. The pellets were dried by air and finally stored at 4°C. A standard method was used to confirm the nutritional profile of test diets (AOAC, 2007) (Table 1).

Table 1. Ingredients of the basal diet and proximate chemical composition (on dry matter basis) (%)

| Ingredients                             | Composition |
|-----------------------------------------|-------------|
| Brown fish meal (65%)^1                 | 30.4        |
| Soybean meal (44%)^1                    | 6.21        |
| Poultry by-product meal^2               | 6.86        |
| Gluten^1                                | 5.95        |
| Wheat bran^1                            | 5.89        |
| Rice bran^1                             | 2.63        |
| Yellow corn^1                           | 2.38        |
| Wheat flour^1                           | 0.63        |
| Fish oil^1                              | 0.21        |
| Vitamin and mineral mix^3               | 0.15        |
| Dicalcium phosphate^4                    | 0.08        |

^1 Supplied by Feed Control Co. Ltd. (Damro, Sidi Salem, Kafrelsheikh, Egypt).
^2 Poultry by-product meal was kindly provided by Elsodor company (Al-Sadat city, Egypt) (Dawood et al., 2020b; Dawood et al., 2020c); fish meal, 60.2% crude protein, 7.9% crude lipid.
^3 Vitamin mixture (mg/kg premix): vitamin A (3300 IU), vitamin D3 (410 IU), vitamin E (2660 mg), vitamin B1 (133 mg), vitamin B2 (580 mg), vitamin B3 (410 mg), vitamin B6 (50 mg), biotin (9330 mg), choline chloride (4000 mg), vitamin C (2660 mg), inositol (330 mg), para-ami-no benzoic acid (9330 mg), niacin (26.60 mg), pantothenic acid (2000 mg); mineral mixture (mg/kg premix): manganese (325 mg), iron (200 mg), copper (25 mg), iodine, cobalt (5 mg).
^4 Dicalcium phosphate, vitamin C, methionine, L-lysine, threonine, tryptophan (DSM in Animal Nutrition & Health, Heerlen, the Netherlands).
^5 Gross energy was calculated based on the values for protein, lipid, and carbohydrate as 23.6, 39.5 and 17.2 kJ/g, respectively.
Fish and experimental protocol

Common carp were collected from a local farm in Kafr El Sheikh governorate and adapted for the laboratory conditions by feeding the basal diet for two weeks (Table 1). Fish with similar weight (4.55±0.03 g) were randomly collected from the stock fish and allocated into 12 glass aquaria (60 L each, four groups/triplicates) at a rate of 15 fish/aquarium. In each aquarium, 50% of the water was replaced daily with fresh dechlorinated water. The tap water was kept overnight in water stock tanks to remove the chlorine. Fish were fed the test diets two times per day (08:00 and 16:00 h) at a rate of 3% for 70 days. The conditions were stable during the trial for water temperature (22.12±0.4°C), pH (7.42–7.75), dissolved oxygen (6.00 mg/L), nitrate-nitrogen (0.04 mg/L), and the photoperiod (12 h light: 12 h darkness).

Sampling schedule

Twelve hours before the final sampling, all fish were starved. To avoid stress during sampling, fish were anesthetized (100 mg/L tricaine methanesulfonate) (Dawood et al., 2020 b). Then, the fish were individually weighed for growth performance calculation. The blood was collected from the caudal vein with a nonheparinized syringe from 3 fish per aquarium. A half of the collected blood was kept in microtube without heparin and allowed to clot for two h to collect serum. While the second half was put in heparinized tubes for phagocytosis analysis. Sera were separated by centrifugation at 3000 rpm for 15 min at 4°C and stored at −20°C until use. Another three fish per aquarium were gently dissected, and the intestines were collected and rinsed in Bouin’s solution for histology sections (Dawood et al., 2019 b; Amin et al., 2019). Another five fish from each aquarium were collected and kept at −20°C for the proximate body composition.

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival were calculated by using the following equations:

\[
WG \% = \frac{(FBW – IBW)}{IBW} \times 100
\]

where:

- \( FBW \) = final body weight (g),
- \( IBW \) = initial body weight (g)

\[
SGR \ (%/day) = 100\left(\frac{\ln FBW – \ln IBW}{T}\right)
\]

where \( T \) is referring to the duration (70 days)

\[
FCR = \frac{FI}{WG}
\]

where \( FI \) = estimated feed intake (g)

\[
PER = \frac{WG}{\text{dry protein intake (g)}}
\]

\[
\text{Survival} \ (%) = \left(\frac{\text{final NO.}}{\text{initial NO.}}\right) \times 100
\]

where \( NO. \) is the number of fish.
Diet and carcass composition
Diet and fish samples were oven-dried at 60°C to constant weight and kept at −20°C for further analysis. A standard method was used for determining chemical composition (moisture, ash, lipids, and crude protein) of the whole fish body and the nutritional profile of test diets (AOAC, 2007).

Intestinal morphology
Standard paraffin embedding procedures were adopted to stain each sample (three cross-sections) using hematoxylin-eosin (Bancroft et al., 1996). Intestinal morphology including the height of villus (from the tip of villus to villus crypt junction), depth of crypt (from the villus crypt junction to the lower limit of the crypt) and the ratio of the height of villus to the depth of crypt (height of villus/depth of crypt) was measured using ImageJ software. A total of six well and random villi and villus-associated crypts were determined for each intestinal cross-section. Goblet cells were stained with Periodic acid–Schiff (PAS) and counted from same-sized villi (n=9), and the results are shown as an average (±SE).

Blood biochemical and immune parameters
Diagnostic commercial kits (Biodiagnostic Co., Egypt) were used for alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides, total serum protein, albumin, urea, uric acid, and creatinine analysis according to manufacturer’s protocol. Globulin content was calculated mathematically. Glucose levels (mg/100 ml) were determined using glucose enzymatic PAP kits obtained from Bio-Merieux (France) (Trinder, 1969).

Serum lysozyme activity was determined by following the turbidimetric assay (Parry et al., 1965). Briefly, the lysozyme substrate consisted of 0.75 mg/ml of gram-positive Micrococcus lysodeikticus lyophilized bacterium cells (Sigma, St. Louis, MO). The units of lysozyme present in blood were expressed as unit/ml. Phagocytic activity was determined according to Kawahara et al. (1991). To calculate the phagocytic index, the number of phagocytic cells was counted in the phagocytic cells according to the following equations:

\[
\text{Phagocytic activity} = \frac{\text{macrophages containing yeast}}{\text{total number of macrophages}} \times 100; \quad \text{phagocytic index} = \frac{\text{number of cells phagocytized}}{\text{number of phagocytic cells}}.
\]

Statistical analysis
Shapiro-Wilk and Levene tests confirmed normal distribution and homogeneity of variance. The obtained data were subjected to one-way ANOVA to evaluate the effect of DME. Differences between means were tested at a 5% probability level using the Duncan test as a post-doc test. The optimum level of DME was calculated by polynomial regression analysis (Yossa and Verdegem, 2015). All the statistical analyses were done via SPSS version 22 (SPSS Inc., IL, USA).
Results

Growth performance
The FBW, WG, SGR, FCR, FI, and PER were influenced by DME in a dose-dependent manner (P<0.05) (Table 2). The polynomial regression equations were as follows: SGR (y = –0.0356x² + 0.1338x + 1.461; R² = 0.8097, the optimal dose = 1.88 g/kg, Figure 1A) and FCR (y = 0.0374x² – 0.1393x + 1.8818; R² = 0.9198, the optimal dose = 1.86 g/kg, Figure 1B). The survival rates were not significantly altered by DME and ranged between 97.78% and 100% (P>0.05).

| Item          | DME (g/kg) | P value |
|---------------|------------|---------|
| IBW (g)       |            |         |
| 0             | 4.52±0.02  | 0.38    |
| 1             | 4.53±0.02  |         |
| 2             | 4.55±0.03  |         |
| 3             | 4.57±0.01  |         |
| FBW (g)       |            |         |
| 0             | 10.92±0.12 a| 0.008   |
| 1             | 11.34±0.15 ab|        |
| 2             | 12.00±0.20 b|         |
| 3             | 11.46±0.12 ab|        |
| WG %          |            |         |
| 0             | 141.79±3.51 a| 0.031   |
| 1             | 150.33±4.39 ab|        |
| 2             | 163.92±5.13 b|         |
| 3             | 150.81±2.96 ab|        |
| SGR (%/day)   |            |         |
| 0             | 1.47±0.02 a| 0.031   |
| 1             | 1.53±0.03 ab|         |
| 2             | 1.62±0.03 b|         |
| 3             | 1.53±0.02 ab|        |
| FI (g/fish)   |            |         |
| 0             | 12.03±0.42 a| 0.021   |
| 1             | 12.26±0.38 ab|        |
| 2             | 12.89±0.18 b|         |
| 3             | 12.47±0.66 ab|        |
| FCR           |            |         |
| 0             | 1.88±0.04 b| 0.041   |
| 1             | 1.80±0.02 a|         |
| 2             | 1.73±0.02 a|         |
| 3             | 1.81±0.03 ab|        |
| PER           |            |         |
| 0             | 1.26±0.02 a| 0.027   |
| 1             | 1.12±0.17 a|         |
| 2             | 1.36±0.03 b|         |
| 3             | 1.32±0.03 b|         |
| Survival (%)  |            |         |
| 0             | 100.00±0.00| 0.596   |
| 1             | 97.78±2.22 |         |
| 2             | 100.00±0.00|         |
| 3             | 97.78±2.22 |         |

*Carcass composition
The body lipid composition was significantly (P=0.0141) improved by feeding DME at 2 g/kg diet when compared to fish fed 0, and 1 g/kg without a difference (P>0.05) with fish fed 3 g/kg diet (Table 3).

| Item          | DME (g/kg) | P value |
|---------------|------------|---------|
| Moisture      |            | 0.946   |
| Crude protein |            | 0.981   |
| Total lipid   |            | 0.0141  |
| Ash           |            | 0.977   |

*Intestinal morphometry
The villus length and the number of goblet cells in the anterior, middle, and posterior intestines were significantly affected by the inclusion of DME in a dose-dependent manner, and the highest value was observed in fish fed 2 g DME/kg diet (P<0.05) (Table 4). The villus width in the middle section of the intestine was significantly increased by 2 g DME/kg (P=0.034) (Table 4).
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Figure 1. Significant quadratic relationships and polynomial regressions analysis (P<0.05) between specific growth rate (A) and feed conversion ratio (B) of common carp and dietary levels of digestive/metabolic enhancer (DME) (g/kg diet). Values are expressed as mean from triplicate groups (n=3)

Table 4. Intestinal morphometry of common carp fed diets containing different levels of digestive/metabolic enhancer (DME) for 70 days*

| Item              | DME (g/kg) | 0       | 1       | 2       | 3       | P value |
|-------------------|------------|---------|---------|---------|---------|---------|
|                   |            | 0       | 1       | 2       | 3       |         |
| Anterior          |            |         |         |         |         |         |
| Villi length (μm) |            | 247.12±15.38 ab | 310.70±37.15 ab | 411.46±13.54 b | 320.65±17.01 ab | 0.007   |
| Villi width (μm)  |            | 101.77±6.63 | 120.70±10.50 | 136.30±15.96 | 165.76±41.73 | 0.322   |
| Intervilli distance (μm) |      | 59.45±10.84 | 65.71±3.55 | 75.95±1.20 | 98.12±9.29 | 0.072   |
| Goblet cells/mm²  |            | 12.67±1.45 a | 14.00±1.73 a | 18.33±0.88 b | 15.33±0.33 ab | 0.045   |
|                  |            |         |         |         |         |         |
| Middle            |            |         |         |         |         |         |
| Villi length (μm) |            | 572.10±51.76 a | 674.67±56.42 a | 1055.16±75.02 b | 676.81±25.93 a | 0.001   |
| Villi width (μm)  |            | 84.67±3.58 a | 110.44±5.43 ab | 119.19±8.60 b | 83.41±12.63 a | 0.034   |
| Intervilli distance (μm) |      | 82.25±10.48 | 96.82±7.73 | 73.20±26.31 | 54.54±1.76 | 0.3      |
| Goblet cells/mm²  |            | 20.33±0.88 a | 21.00±1.53 ab | 26.67±1.45 b | 20.00±1.16 a | 0.019   |
|                  |            |         |         |         |         |         |
| Posterior         |            |         |         |         |         |         |
| Villi length (μm) |            | 117.98±24.17 a | 276.48±88.98 ab | 306.22±20.31 b | 243.51±16.43 ab | 0.046   |
| Villi width (μm)  |            | 116.18±5.49 | 221.02±66.13 | 112.15±11.09 | 133.93±17.73 | 0.172   |
| Intervilli distance (μm) |      | 87.87±15.46 | 138.36±42.51 | 74.81±7.20 | 112.06±14.80 | 0.324   |
| Goblet cells/mm²  |            | 4.67±0.67 a | 6.33±0.33 ab | 7.67±0.88 b | 6.33±0.33 ab | 0.047   |

*Values expressed as means ± SE (n = 3). Different letters indicate significant differences for each pairwise comparison between treatments.
The polynomial regression equations were as follows: anterior villus length ($y = -38.598x^2 + 147.93x + 235.68; R^2 = 0.8096$, the optimal dose $= 1.92$ g/kg, Figure 2A) and number of goblet cells ($y = -1.8333x^2 + 5.9667x + 19.467; R^2 = 0.4917$, the optimal dose $= 1.63$ g/kg, Figure 2B).

![Graph A: Anterior villus length vs DME](image)

![Graph B: Number of goblet cells vs DME](image)

Figure 2. Significant quadratic relationships and polynomial regressions analysis ($P<0.05$) between anterior villi length (µm) (A) and middle intestine number of goblet cells (B) of common carp and dietary levels of digestive/metabolic enhancer (DME) (g/kg diet). Values are expressed as mean from triplicate groups ($n=3$)

Generally, fish fed DME exhibited increased length, branching, and integrity with regular structure, muscular, serosa, submucosa, and intact tunica mucosa (Figure 5).

**Blood biochemistry**

The blood biochemical parameters were insignificantly affected by DME inclusion ($P>0.05$), except for blood AST, ALT, albumin, globulin, and total protein (Table 5). Fish fed DME at 2 g/kg displayed low AST and ALT values ($P = 0.025$ and $P = 0.043$) and increased total protein and globulin ($P = 0.002$ and $P = 0.003$). Additionally, fish fed 2 and 3 g DME/kg levels displayed significantly increased albumin levels ($P = 0.006$).
Figure 3. Significant quadratic relationships and polynomial regressions analysis (P<0.05) between blood alanine aminotransferase (ALT) (A), lysozyme activity (B), and phagocytic activity (C) of common carp and dietary levels of digestive/metabolic enhancer (DME) (g/kg diet). Values are expressed as mean from triplicate groups (n=3)
Figure 4. Changes in lysozyme activity (A), phagocytic activity (B), and phagocytic index (C) of common carp fed on dietary levels of digestive/metabolic enhancer (DME) (g/kg diet). Values are expressed as mean from triplicate groups (n=3). Bars assigned by different letters are significantly different at P<0.05.

Table 5. Blood biochemical parameters of common carp fed diets containing different levels of digestive/metabolic enhancer (DME) for 70 days*

| Item           | DME (g/kg) | P value |
|----------------|------------|---------|
|                | 0         | 1       | 2       | 3         |
| Glucose (mg/dl)| 13.80±0.13| 14.08±0.11| 14.29±0.20| 14.20±0.23| 0.277    |
| Total cholesterol (mg/dl) | 73.26±0.65| 68.54±0.58| 63.87±1.48| 70.47±0.44| 0.051    |
| Triglyceride (mg/dl) | 123.45±2.46| 127.08±4.58| 136.58±3.25| 133.24±2.15| 0.077    |
| ALT (U/l)      | 11.11±3.53 b| 5.40±1.41 ab| 4.01±0.13 a| 5.24±1.36 ab| 0.025    |
| AST (U/l)      | 117.56±18.69 b| 83.25±13.21 ab| 74.65±2.81 a| 78.65±8.13 ab| 0.043    |
| Total protein (g/dl) | 4.73±0.07 a| 4.92±0.04 a| 5.30±0.10 b| 5.03±0.05 ab| 0.002    |
| Albumin (g/dl) | 3.31±0.05 a| 3.45±0.02 ab| 3.57±0.03 b| 3.60±0.06 b| 0.006    |
| Globulin (g/dl) | 1.42±0.06 a| 1.47±0.03 a| 1.73±0.06 b| 1.43±0.01 a| 0.003    |
| Urea (mg/dl)   | 5.87±0.12| 5.18±0.07| 4.73±0.23| 5.14±0.08| 0.053    |
| Creatinine (mg/dl) | 0.31±0.01| 0.29±0.00| 0.25±0.02| 0.30±0.01| 0.051    |
| Uric acid (mg/dl) | 2.06±0.03| 1.95±0.02| 1.84±0.03| 1.85±0.03| 0.061    |

*Values expressed as means ± SE (n=3).
The polynomial regression equation for ALT was \( y = 1.7358x^2 - 7.1092x + 11.029; R^2 = 0.9953; \) the optimal dose = 2.05 g/kg (Figure 3A).

**Immunological parameters**

Dietary DME increased the phagocytic and lysozyme activities in a dose-dependent manner \( (P<0.05) \) (Figures 4A and 4B). Lysozyme and phagocytic activities were increased by feeding DME at 2 g/kg diet, while the phagocytic index increased by 2 and 3 g/kg diet (Figure 4C).

![Intestinal morphology](image)

Figure 5. Intestinal morphology of fish fed the test diets (H&E, X100). 1) The intestine of fish fed the control diet showing normal features of intestinal villi in the three segments (foregut, midgut, and hindgut). 2) The intestine of fish fed on digestive/metabolic enhancer (DME) showing an increase in the density of intestinal villi. 3) The intestine of fish fed on digestive/metabolic enhancer (g/kg diet) showing an increase in the length and branching intestinal villi (arrow) (foregut, midgut, and hindgut) in a dose dependent manner
The polynomial regression equations were as follows: lysozyme activity \( y = -3.01x^2 + 11.175x + 31.261; R^2 = 0.7393 \), the optimal dose = 1.86 g/kg, Figure 3B), phagocytic activity \( y = -2.6742x^2 + 9.7662x + 28.636; R^2 = 0.7503 \), the optimal dose = 1.63 g/kg, Figure 3C).

**Discussion**

The selection of feed additives is one of the most critical factors for the formulation and commercial production of aquafeeds (Dawood and Koshio, 2019; El Basuini et al., 2020). Among them, feed additives have the potential to raise the protein-sparing in the fish body without reducing the feed utilization and health condition, which result in reducing the cost of feed (Lin and Wu, 2014; Hoseini et al., 2018). The results indicated that DME improved the protein and feed efficiency and, in turn, the growth performance.

The DME used in the current study includes both MCFA and taurine. Taurine is sufficiently presented in fish meal; therefore, low fish meal diets must be supplemented with taurine because fish cannot synthesize taurine in their bodies (Koven et al., 2016; Michelato et al., 2018; Abdel-Tawwab and Monier, 2018). In the current study, the fish meal was included in the basal diet at a low amount (2%) to balance the nutritional value and as a natural attractant. Taurine is proposed to induce growth performance by enhancing the utilization of amino acids and protein efficiency (Yan et al., 2019). The obtained data implied that DME supplementation improved the feed intake and protein efficiency ratio, which can be attributed to the role of taurine in improving the protein utilization in carp’s intestine. Similarly, taurine improved the feed efficiency and growth performance in common carp (Cyprinus carpio L.), Nile tilapia (Oreochromis niloticus), and grass carp (Ctenopharyngodon idella) (Michelato et al., 2018; Al-Feky et al., 2016 a; Shen et al., 2017; Al-Feky et al., 2016 b; Yan et al., 2019; Abdel-Tawwab and Monier, 2018). The DME mixture also contains MCFA, which has been reported to improve feed efficiency and, in turn, the growth performance (Rimoldi et al., 2018). Taurine or MCFA has been reported to induce the utilization of fish feed through increasing the absorption by intestinal cells (Rimoldi et al., 2018; Shen et al., 2019; Hong et al., 2004).

The measurements of the intestinal morphometric indices are usually used to give a clear view of the absorption capacity of nutrients through the villi (Rašković et al., 2011; El Asely et al., 2020). The villus length and the number of goblet cells in the anterior, middle, and posterior intestines were influenced by the inclusion of DME, especially in fish fed 2 g DME/kg diet. This result is further giving a possible reason for the improved feed efficiency in common carp fed DME in the current study. It has been reported that the efficiency of feed absorption is relying on the villi surface area (width and length) (Caspary, 1992; Abdel-Tawwab and Monier, 2018; Adeshina et al., 2019). MCFA can improve the function of fish’s gastrointestinal tract (GIT) to digest diets efficiently by inducing the GIT absorptive capacity (Hong et al., 2004; Rimoldi et al., 2018). The goblet cells protect the mucosal layer of the intestine by the secretion of mucus and antibacterial substances which improve digestion and
Effects of MCF A and taurine in common carp

decrease the number of pathogenic bacteria (Noga, 1996; Pirarat et al., 2015). The results indicated that using DME resulted in an increased number of goblet cells, which confirm the beneficial role of DME in the common carp diet.

Fish fed DME at 2 g/kg displayed low AST and ALT values and increased total protein, albumin, and globulin levels. The improved blood total protein, albumin, and globulin values indicated that fish are immunologically healthy due to feeding with nutritionally balanced diets (Dawood, 2016). Additionally, the inclusion of DME reduced the levels of ALT and AST with regards to the control. ALT and AST are always used to refer to the condition of the liver, and high levels mean that the liver is suffering from toxic or harmful conditions (Dawood et al., 2020 e). Similarly, it has been reported that dietary taurine or MCFA can increase the blood proteins in Nile tilapia (O. niloticus) and yellow catfish (Pelteobagrus fulvidraco) (Zhang et al., 2018; Abd El-Naby et al., 2019). Nevertheless, the blood glucose, total cholesterol, and triglyceride levels displayed non-significant differences among the groups which contradicted the findings of Hoseini et al. (2018), who reported that Persian sturgeon (Acipenser persicus) exhibited decreased glucose levels and increased total cholesterol and triglyceride levels by taurine feeding. The difference in the obtained results can be attributed to the species-specific effects, the interaction effect of MCFA and taurine, and the experimental conditions.

The results of the present study also showed a rise in immune parameters (lysozyme and phagocytic activities). Lysozyme activity can induce the immune system through anti-inflammatory and bactericidal properties against gram-positive and gram-negative bacteria (Ángeles Esteban, 2012; Saeidi asl et al., 2017). In the present study, fish fed both taurine and MCFA displayed increased lysozyme activity. Also, the serum phagocytic activity and index were increased in this study, which indicates the enhancement of the humoral immune system of fish fed DME. Taurine and MCFA are suggested to have a role in the immune regulation by stimulating the anti-inflammatory and pro-inflammatory gene expressions in the GIT (Wang et al., 2006; Zhou et al., 2019; Yan et al., 2019). The present study also stated that the phagocytic activity of common carp fed DME was higher than that of the control fish. The efficacy of taurine and MCFA used in fish or shellfish culture through protection against infectious pathogens is often attributed to elevated immune responses (Salsali et al., 2008; Rimoldi et al., 2018).

**Conclusion**

Based on the beneficial effects of DME on the growth, feed utilization, and immune response, it can be concluded that the inclusion of DME is highly recommended to improve the performance of common carp fed diets with low fish meal content. The optimal supplementation based on the obtained results ranges from 1.63 to 2.05 g DME per kg diet.

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