Research Article

Effect of Dietary Combination of Methionine and Fish Oil on Cellular Immunity and Plasma Fatty Acids in Infectious Bursal Disease Challenged Chickens

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This study was carried out to investigate the modulatory effects of dietary methionine and fish oil on immune response, plasma fatty acid profile, and blood parameters of infectious bursal disease (IBD) challenged broiler chickens. A total of 300 one-day-old male broiler chicks were assigned to one of six dietary treatment groups in a 3 × 2 factorial arrangement. There were three levels of fish oil (0, 2.5 and 5.5%), and two levels of methionine (NRC recommendation and twice NRC recommendation). The results showed that the birds fed with 5.5% fish oil had higher total protein, white blood cell count, and IL-2 concentration than those of other groups at 7 days after IBD challenge. Inclusion of fish oil in diet had no effect on IFN-γ concentration. However, supplementation of methionine twice the recommendation enhanced the serum IFN-γ and globulin concentration. Neither of fish oil nor methionine supplementation affected the liver enzymes concentration. It can be suggested that a balance of moderate level of fish oil (2.5%) and methionine level (twice NRC recommendation) might enhance immune response in IBD challenged broiler chickens.

1. Introduction

The strategies to enhance immune-functional abilities through nutrition have extended to poultry nutrition in the last decade. Today, polyunsaturated fatty acids (PUFA) are widely accepted as a part of modern nutrition, because of their beneficial health-promoting effect in animal and human diets [1, 2]. Several empirical studies have shown that modification of dietary fatty acids alters the fatty acid composition of tissue lipids [3–5] and has notable effect on the inflammatory immune response [2, 6–9]. These modulations are regulated through bioactive lipid mediators such as eicosanoids, prostaaglandins, leukotrienes, lipoxins, and resolvins [10]. Interestingly, it has been reported that n-3 PUFA intake may attenuate the growth-inhibitory effects of proinflammatory cytokine in various species [9, 11–13]. Moreover, inclusion of fish oil as a source of precursors for eicosanoids in the diet appears to improve humoral immunity and ameliorate the suppression of the cellular immune response caused by prostaglandin E2 (PGE2) [14, 15].

On the other hand, methionine as an essential amino acid is linked to PUFA metabolism. The methionine-homocysteine cycle produces methyl groups for the synthesis of phosphatidylcholine from phosphatidylethanolamine [16, 17]. Phosphatidylcholine is essential for the delivery of PUFA from the liver to the plasma and tissues. In an early study, Tidwell [18] found that fat absorption increased when lipotropic amino acid such as methionine was ingested along with the lipids. It has also been shown that high methionine
supplementation (224 mg/kg body weight) increases docosa-
hexaenoic acid in the liver and jejunum [19]. Meanwhile, S-
adenosylmethionine, a product of methionine metabolism,
plays an important role as the methyl group donor in
transmethylation reactions, in which the synthesis of mem-
brane phospholipids (particularly phosphatidylcholine) is
necessary for the maintenance of membrane fluidity [20].
Accordingly, it is reasonable to expect a more effective
necessary for the maintenance of membrane fluidity [20].

2. Materials and Methods

2.1. Birds and Housing. A total of 300 one-day-old male
broiler chicks (Cobb) were purchased from a local hatchery.
The chicks were individually wing-banded, weighed, and
housed in cages in the open sided house with cyclic tem-
perature (minimum, 24°C; maximum, 34°C). The relative
humidity was between 80 and 90%. Feed and water were
provided ad libitum and lighting was continuous.

2.2. Experimental Design. Experimental procedure was
approved by the (ACUC) Animal Care and Use Committee
of Universiti Putra Malaysia. Commencing from day one,
five replicate cages of 10 chicks each were assigned to one of
the six dietary treatments, giving a total of 30 pens. The diets
were formulated to meet or exceed the requirements of the
National Research Council (NRC, 1994) for broilers of this
age [21]. There were three levels of tuna oil (0, 2.5, and 5.5%)
and two levels of DL-methionine (NRC recommendation
and twice NRC recommendation). Therefore, the following
six dietary treatments were compared: (1) basal diet based
on NRC recommendation (M1F0); (2) basal diet containing
methionine 2 times higher than NRC (M1F2); (3) basal diet
containing 2.5% tuna oil + 3.5% sunflower oil (M1F2S3); (4)
basal diet containing 5.5% tuna oil + 0.5% sunflower oil
(M2F3S5); (5) combination of diets 2 and 3 (M2F5S3); (6)
combination of diets 2 and 4 (M2F8S3) (Tables 1 and
2). The choice of tuna oil in our study was based on the
commercial availability of oil in large scale and the higher
level of docosahexaenoic acid (DHA) compared with other
fish oil sources. To prevent lipid peroxidation, precautions
were taken by mixing feed every two weeks and addition of
butylated hydroxytoluene (BHT) and ethoxyquin (EQ) as
antioxidants (100 g/ton) to diets.

2.3. Chemical Analysis. The proximate chemical analysis
of the feeds was carried out following standard methods of
AOAC (2000) [22]. The dry matter was determined by oven
drying in a forced-air oven for 24 h at 105°C. The Kjeltec
Auto Analyzer (Tecator, Hoganas, Sweden) was used to
determine nitrogen and then converted to crude protein (CP
= N × 6.25), while the ether extract (EE) was determined in

petroleum ether using a 2025 Soxtec Auto Analyzer (Tecator,
Hoganas, Sweden). The ash content was determined by ashing
the samples in a muffle furnace at 550°C for 4 h.

2.4. Amino Acid Composition of Diets. Amino acids were
analyzed by hydrolyzing samples (0.2 g) with 5 mL of 6 N
HCl at 110°C for 24 hours in sealed evacuated tubes to obtain
hydrolyzate suitable for analyzing all amino acids except
methionine and cysteine [23]. An internal standard was then
added into the cooled hydrolyzate which was diluted with
detonized water as well as 10 μL of this filtrate as mixed
with 70 μL of AccQ-Fluor borate buffer and 20 μL of AccQ-
Fluor reagent. Then the samples were analyzed by using
high-performance liquid chromatography (HPLC) with a
Waters 717 Plus HPLC autosampler and a Waters 2475 multi-
λ fluorescence detector set at an excitation wavelength of
250 nm and an emission wavelength of 395 nm. Separation
was achieved in a Waters AccQ-Tag amino acid analysis
column, 3.9 × 150 mm at a flow rate of 1 mL/min (Waters
Corporation, Milford, MA, USA). Cystine and methionine
were analyzed as cystic acid (Cya) and methionine sulphone
(MetO2), respectively, by oxidation with performic acid for
16 h at 4°C and neutralization with hydrobromic acid before
hydrolysis.

2.5. Challenge Protocol. The clinical form of IBD usually
occurs in chickens from 3 to 6 weeks of age. Thus, on day
28 of age, all birds were challenged orally with commercial
live IBD vaccine (V877 strain, Malaysian Vaccines and
Pharmaceuticals Sdn. Bhd). The live vaccine was chosen to
induce infection in the birds. The strain was characterized
as an intermediate classical strain which is used under
normal conditions as a standard procedure applying for most
situations in the field. Each bird was inoculated with a dose of
10⁶⁰ EID₅₀ IBD viruses into the lumen of the crop by oral
gavage [24].

2.6. Fatty Acid Analysis. The total fatty acids were extracted
from diets and plasma samples using chloroform: methanol
2:1 (v/v) based on the method by Folch et al. [25] and
modified by Ebrahimi et al. [26] with an addition of antiox-
idant (0.2 mg/L BHT) to prevent oxidation during sample
preparation. The experimental diets and plasma were mixed
in 40 mL chloroform: methanol (2:1 v/v). Transmethylation
of the extracted fat to fatty acid methyl esters (FAME) were
carried out using KOH in methanol and 14% methanolic
boron trifluoride (BF₃) (Sigma Chemical Co. St. Louis,
Missouri, USA) according to the methods in AOAC (2000).
The methyl esters were quantified by gas chromatography
(Agilent 7890A) using a 30 m × 0.25 mm ID (0.20 μm film
thickness) Supelco SP-2330 capillary column (Supelco, Inc.,
Belleville, PA, USA). One microliter of FAME was injected
into an autosampler into the chromatograph, equipped with a
split/splitless injector and a flame ionization detector (FID).
The injector temperature was programmed at 250°C, and the
detector temperature was 300°C. The column temperature
program was initiated to run at 100°C, for 2 min, warmed
to 170°C at 10°C/min, held for 2 min, warmed to 220°C at
Table 1: Ingredients and nutrients composition of experimental diets.

| Ingredient (%) | Starter (1 to 21 d) | Finisher (22 to 42 d) |
|----------------|-------------------|---------------------|
|                | M₁F₀ | M₂F₀ | M₁F₂ | M₂F₂ | M₁F₃ | M₂F₃ | M₁F₅ | M₂F₅ | M₁F₀ | M₂F₀ | M₁F₂ | M₂F₂ | M₁F₃ | M₂F₃ | M₁F₅ | M₂F₅ |
| Corn           | 44.91 | 44.61 | 45.61 | 45.61 | 45.86 | 45.61 | 49.90 | 49.59 | 51.45 | 51.14 | 51.55 | 51.14 | 49.90 | 49.59 | 51.45 | 51.14 |
| Soybean meal   | 43.85 | 43.60 | 43.73 | 43.18 | 43.48 | 38.67 | 38.47 | 38.43 | 38.23 | 38.33 | 38.23 | 38.43 | 38.23 | 38.43 | 38.23 |
| Palm oil       | 6.58  | 6.58  | 0.00  | 0.00  | 0.00  | 7.31  | 7.31  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| Sunflower oil  | 0.00  | 0.00  | 3.50  | 3.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  |
| Tuna oil       | 0.00  | 0.00  | 2.50  | 2.50  | 5.50  | 5.50  | 2.50  | 2.50  | 5.50  | 5.50  | 2.50  | 2.50  | 5.50  | 5.50  |
| Dicalcium phosphate | 1.91 | 1.91  | 1.91  | 1.91  | 1.91  | 1.91  | 1.77  | 1.77  | 1.77  | 1.77  | 1.77  | 1.77  | 1.77  | 1.77  |
| Limestone      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.06  | 1.06  | 1.06  | 1.06  | 1.06  | 1.06  | 1.06  |
| Salt           | 0.44  | 0.44  | 0.44  | 0.44  | 0.44  | 0.44  | 0.31  | 0.31  | 0.31  | 0.31  | 0.31  |
| Vitamin premix¹ | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  |
| Mineral premix¹ | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  |
| DL-Methionine  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  |
| Lysine         | 0.26  | 0.26  | 0.26  | 0.26  | 0.26  | 0.26  | 0.26  | 0.26  |

Calculated composition

- **Crude protein**: 22.00 22.00 22.00 22.00 22.00 22.00 20.50 20.50 20.50 20.50 20.50 20.50
- **ME (Kcal/kg)**: 3080 3080 3080 3080 3080 3080 3150 3150 3150 3150 3150 3150
- **Available phosphorus**: 0.45 0.45 0.45 0.45 0.45 0.45 0.42 0.42 0.42 0.42 0.42 0.42
- **Calcium**: 1.00 1.00 1.00 1.00 1.00 1.00 0.90 0.90 0.90 0.90 0.90 0.90
- **Methionine**: 0.55 1.1 0.55 1.1 0.55 1.1 0.50 1.00 0.50 1.00 0.50 1.00
- **Lysine**: 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20 1.20
- **Na**: 0.20 0.20 0.20 0.20 0.20 0.20 0.20 0.20 0.20 0.20 0.20 0.20

Analyzed composition

- **Methionine**: 0.59 1.14 0.63 1.02 0.64 1.06 0.58 0.84 0.61 0.91 0.59 0.90
- **Lysine**: 1.26 1.28 1.19 1.29 1.22 1.19 1.11 1.01 1.05 1.13 1.09 1.10
- **Cysteine**: 0.42 0.34 0.40 0.38 0.47 0.37 0.37 0.35 0.34 0.26 0.35 0.40
- **DM**: 89.60 89.91 89.44 89.80 89.41 89.64 89.95 89.86 90.11 90.45 89.46 89.74
- **ASH**: 7.83 7.64 7.70 8.06 7.51 7.11 7.76 8.24 7.01 8.03 7.14 7.78
- **CP**: 7.50 3.11 3.64 2.21 3.44 3.68 4.32 4.44 2.95 3.17 3.47 2.50
- **CF**: 7.50 3.11 3.64 2.21 3.44 3.68 4.32 4.44 2.95 3.17 3.47 2.50

¹Supplied per kilogram of diet: vitamin A: 1,500IU; cholecalciferol: 200IU; vitamin E: 10IU; riboflavin: 3.5mg; pantothenic acid: 10mg; niacin: 30mg; cobalamin: 10µg; choline chloride: 1,000mg; biotin: 0.15mg; folic acid: 0.5mg; thiamine: 1.5mg; pyridoxine: 3.0mg; iron: 80mg; zinc: 40mg; manganese, 60mg; iodine: 0.18mg; copper: 8mg; selenium: 0.15mg; BHT + EQ: 100mg.

7.5°C/min, and then held for 20 min to facilitate optimal separation. All results of fatty acid were presented as the percentage of total fatty acids. All peaks were quantified using fatty acid standards (Supelco 18919, fatty acid methyl ester mixture, USA).

2.7. Serum Chemistry and Total White Blood Cell Count. On day 28 (before challenge), 35 (7 days after challenge), and 42 (14 days after challenge), five birds from each treatment groups were randomly chosen and their blood samples (3.0mL) were collected from the brachial vein using a 23-gauge needle. Five different birds were used each time for sampling. The blood samples were immediately aliquoted into non-anticoagulant and anticoagulant tubes containing K-EDTA as an anticoagulant. Blood in the nonanticoagulant tubes was allowed to clot for 2h at 37°C, and then the serum was decanted [27]. The blood samples in the anticoagulant tubes were packed on ice until they were centrifuged (3000 g for 15 min). Serum and plasma were stored at −20°C until analysis. Serum total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, and triglyceride were measured by specific commercial kits (Roche Diagnostica, Basel, Switzerland) using an autoanalyzer (Hitachi 902 automatic autoanalyzer). Total WBC counts were determined using an automated hematological analyzer (Cell-Dyn 3700; Abbott Laboratories, Abbott Park, IL, USA).

The IL-2 and IFN-γ levels in the serum were measured using chicken ELISA kit (Cusabio Biotech, CA, USA) and microplate reader (Bio-Tek Instruments Inc. ELX 800;
2.8. Statistical Analysis. Data were analyzed using the GLM procedure of SAS [30]. Data were subjected to 2-way ANOVA in a 3 × 2 factorial arrangement with fish oil and DL-methionine as the main effects and their interactions. When interactions were significant, a separate ANOVA was conducted within each main effect. Significant differences were separated using Duncan's multiple range tests. The results were expressed as mean ± SEM. Statistical significance was considered at \( P < 0.05 \).

3. Results

Fatty acid composition analysis of plasma showed that there is no significant interaction between dietary methionine and fish oil (Table 3). However, supplementation of fish oil
|       | Before challenge | 7 d after challenge | 14 d after challenge |
|-------|------------------|---------------------|---------------------|
|       | WBC (10^9/L)     | ALB (g/L)           | TP (g/L)            | GLU (g/L)       | WBC (10^9/L) | ALB (g/L) | TP (g/L) | GLU (g/L) | WBC (10^9/L) | ALB (g/L) | TP (g/L) | GLU (g/L) |
| F_0   | 17.6 ± 1.7^c     | 11.7 ± 0.7          | 23.1 ± 1.1          | 11.4 ± 0.9^b     | 40.4 ± 6.6^b   | 10.3 ± 1.0  | 22.5 ± 1.2^b | 10.5 ± 1.2  | 49.0 ± 5.5   | 12.2 ± 1.2  | 28.1 ± 1.4 | 19.0 ± 1.7 |
| F_2.5 | 32.4 ± 1.7^a     | 12.4 ± 0.7          | 21.0 ± 1.1          | 9.0 ± 0.8^b      | 72.8 ± 7.4^b   | 10.5 ± 1.0  | 23.3 ± 1.1^b | 10.8 ± 1.0  | 54.6 ± 6.2   | 14.9 ± 1.1  | 32.8 ± 1.2 | 19.4 ± 1.4 |
| F_5.5 | 40.2 ± 2.0^a     | 10.0 ± 0.7          | 23.4 ± 1.1          | 15.9 ± 1.0^c     | 80.4 ± 8.0^c   | 12.4 ± 0.9  | 25.8 ± 1.1^c | 12.4 ± 0.9  | 54.5 ± 6.3   | 12.4 ± 1.2  | 29.3 ± 1.3 | 16.9 ± 1.6 |
| M_1   | 31.8 ± 1.3       | 10.3 ± 0.5          | 23.7 ± 0.9          | 12.7 ± 0.7       | 55.3 ± 6.2     | 11.8 ± 0.8  | 21.7 ± 0.9  | 9.3 ± 0.9^c | 48.4 ± 5.1   | 13.8 ± 0.9  | 29.0 ± 1.1 | 18.4 ± 1.3 |
| M_2   | 28.8 ± 1.5       | 11.9 ± 0.5          | 22.8 ± 0.9          | 12.3 ± 0.8       | 68.9 ± 5.8     | 10.5 ± 0.8  | 23.9 ± 0.9  | 13.8 ± 0.8^c | 59.2 ± 7.1   | 12.1 ± 1.0  | 29.5 ± 1.0 | 18.3 ± 1.2 |

**ANOVA (P value)**

|       | F     | M     | F × M  |
|-------|-------|-------|--------|
|       | 0.0001| 0.15  | 0.14   |
|       | 0.32  | 0.49  | 0.97   |
|       | 0.003 | 0.49  | 0.97   |
|       | 0.002 | 0.18  | 0.45   |
|       | 0.22  | 0.45  | 0.61   |
|       | 0.04  | 0.12  | 0.52   |
|       | 0.01  | 0.26  | 0.36   |
|       | 0.96  | 0.35  | 0.54   |
|       | 0.27  | 0.24  | 0.51   |
|       | 0.98  | 0.12  | 0.30   |
|       | 0.85  | 0.44  | 0.16   |

^a,b,cMeans ± SEM within a column subgroup with no common letters differ at P < 0.05.

WBC: total white blood cell; TP: total protein; ALB: albumin; GLU: globulin.

F_0: 0% fish oil; F_2.5: 2.5% fish oil; F_5.5: 5.5% fish oil.

M_1: methionine (NRC level); M_2: methionine (2-fold of NRC).
increased plasma \( n-3 \) PUFA level \( (P < 0.05) \) and decreased \( n-6/n-3 \) compared to the control group. Methionine supplementation twice the recommended level was not affected the plasma fatty acids profile \( (P > 0.05) \).

No significant interaction was observed for total white blood cell, plasma total protein, albumin, and globulin throughout the study (Table 4). Before challenge and 7 days after challenge, birds of \( F_{5.5} \) group had significantly higher total WBC than \( F_{2.5} \) and \( F_0 \) birds. These birds had also higher total protein at before challenge period and higher total protein at 7 days after challenge than the other two groups \( (P < 0.05) \). At 7 days after challenge, the concentration of globulin was significantly higher in \( M_2 \) group than \( M_1 \). At 14 days after challenge, there were no differences between treatment groups for all the parameters measured in this study, and it seems that the birds were fully recovered from the IBD challenge by this time. In addition, the concentration of liver enzymes, cholesterol, and triglyceride in serum was not influenced by methionine or fish oil supplementation in both prechallenge and 14 days postchallenge periods (Tables 5 and 6).

The effects of fish oil and methionine supplementation on serum IL-2 and IFN-\( \gamma \) are shown in Table 7. Regardless of methionine supplementation, the concentration of IL-2 was higher \( (P < 0.05) \) in \( F_{5.5} \) birds compared to \( F_0 \) and \( F_{2.5} \) at 7 days after challenge. There were significant interactions between dietary fish oil and methionine for IL-2 at 2 days after challenge and IFN-\( \gamma \) at 7 days after challenge. Comparison of the interaction effect was revealed that only the birds of \( M_2 \) group which were supplemented with fish oil had lower serum IL-2 at 2 days after challenge \( (P < 0.05) \) (Table 8). However, methionine supplementation at twice the recommendation was increased IFN-\( \gamma \) concentration only in birds with no fish oil supplementation \( (M_1 F_0) \). On the other hand, these groups of birds \( (F_{2.5} \) and \( F_{5.5} \) and showed lower concentration of IFN-\( \gamma \) only when they were supplemented with twice the methionine recommendation.

### 4. Discussion

Dietary \( n-3 \) PUFA enrichment alters the fatty acid profile of plasma and meat towards higher level of long chain PUFA [31–33]. In agreement, as indicated by current study results, the total \( n-3 \) PUFA, EPA, and DHA of plasma significantly increased with inclusion of fish oil in diet. In addition, our results are consistent with Khalifa et al. [34] showing that the dietary \( n-3 \) PUFA enrichment decreases the proportion of arachidonic acid \((C20: 4n-6)\) in chicken plasma. It has been shown that the fatty acid composition of phospholipid fraction of plasma is closely related to the fatty acid composition of erythrocyte and platelet membrane phospholipids [35]. Therefore, plasma phospholipid fatty acids have the potential to function as a surrogate measure of the potential effects of diet on the whole range of cell membrane lipids. This noninvasive measure may facilitate the short or long term dietary fatty acid modulation studies in the chicken model. The addition of fish oil also may improve the absorption of fat-soluble vitamins, decrease palaverulence, increase palatability, reduce the rate of food passage, and allow a better absorption of all nutrients present in the diet [36]. The dietary supplementation of fish oil increased the number of WBC in the peripheral blood, indicating an immune-stimulatory effect of \( n-3 \) essential fatty acids. This finding coincides with the report of Mansoub [37] that feeding high \( n-3 \) diet increased WBC count, total protein, and globulin.

Regarding the immunological challenge of the study, an activation of immune system was observed, as indicated by the lower serum IL-2 and higher IFN-\( \gamma \) concentration at 2 days after challenge compared to before challenge condition. The \( n-3 \) fatty acids and fish oil are generally known to decrease the levels of proinflammatory cytokines such as IL-1, IL-2, IL-6, and TNF-\( \alpha \) [38–40]. It has been reported that \( n-3 \) PUFA supplementation increased T-cell proliferation and enhanced IL-2 production by splenocytes in mice [41].
Table 7: Effect of fish oil and methionine supplementation on serum IL-2 and IFN-γ levels (pg/mL) in broiler chickens challenged with IBD.

|                | IL-2 Before challenge | IL-2 2 d after challenge | IL-2 7 d after challenge | IFN-γ Before challenge | IFN-γ 2 d after challenge | IFN-γ 7 d after challenge |
|----------------|-----------------------|--------------------------|--------------------------|------------------------|--------------------------|--------------------------|
| F0             | 0.452 ± 0.008         | 0.365 ± 0.002b           | 0.428 ± 0.002b           | 0.99 ± 0.07            | 1.38 ± 0.05              | 1.10 ± 0.04b             |
| F2.5           | 0.442 ± 0.009         | 0.365 ± 0.003            | 0.423 ± 0.002b           | 0.98 ± 0.07            | 1.35 ± 0.05              | 0.87 ± 0.04b             |
| F5.5           | 0.438 ± 0.008         | 0.374 ± 0.002            | 0.447 ± 0.002b           | 0.95 ± 0.06            | 1.39 ± 0.05              | 0.92 ± 0.04b             |
| M1             | 0.440 ± 0.007         | 0.373 ± 0.002a           | 0.435 ± 0.002            | 1.06 ± 0.06            | 1.37 ± 0.04              | 0.93 ± 0.03b             |
| M2             | 0.448 ± 0.007         | 0.361 ± 0.001b           | 0.432 ± 0.001            | 0.95 ± 0.05            | 1.37 ± 0.04              | 1.06 ± 0.03b             |

ANOVA (P value)

F 0.57 0.051 0.006 0.88 0.76 0.002
M 0.41 0.008 0.54 0.49 1.00 0.04
F × M 0.18 0.014 0.10 0.26 0.14 0.01

Mean ± SEM within a column subgroup with no common letters differ at P < 0.05.

It has also shown that PUFA deficiency may reduce the lymphocyte proliferation, IL-2 production, monocyte, and polymorphonuclear cell chemotaxis in mammals [34, 42, 43]. Consistently, Sijben et al. [15] showed that IL-2 expression enhanced in lipopolysaccharide- (LPS-) injected birds fed fish oil rich diet [44]. Similarly, in our study, supplementation of fish oil enhanced IL-2 response and suppressed IFN-γ level. This immune-modulating effects from feeding diets rich in n-3 PUFA may be explained by the capacity of the n-3 PUFA to reduce prostaglandin E (PGE) production through competition with arachidonic acid as a substrate for cyclooxygenase [10]. In infections, reduction of PGE stimulates immunity by increasing TNF [45] and IL-2 [2]. However, the reduction of IFN-γ level and consequently inflammation and immune response in fish oil supplemented birds are not clear and may not be explained by this mechanism. The fact that this reduction is only observed in the birds with high methionine supplementation may shed some light on this issue. Previous studies showed that high consumption of diet rich in n-3 PUFA may be explained by the capacity of the n-3 PUFA to reduce prostaglandin E (PGE) production through competition with arachidonic acid as a substrate for cyclooxygenase [10]. In infections, reduction of PGE stimulates immunity by increasing TNF [45] and IL-2 [2]. However, the reduction of IFN-γ level and consequently inflammation and immune response in fish oil supplemented birds are not clear and may not be explained by this mechanism. The fact that this reduction is only observed in the birds with high methionine supplementation may shed some light on this issue. Previous studies showed that high consumption of diet rich in DHA increased methionine adenosyltransferase (MAT) activity and upregulated MAT mRNA expression in transmethylation metabolic pathway of methionine. The resultant increase in S-adenosylmethionine synthesis by MAT stimulates S-adenosylhomocysteine production, with the consequential upregulation of cystathionine β-synthase and cystathionineγ-lyase, and as a result, removal of methionine permanently by converting it to cysteine [46–48]. Therefore, it may be speculated that the low dietary level of methionine impaired immune response and resulted in lower synthesis of IgG antibodies or perhaps thymus derived T-helper cells function [49, 50]. Pathologically also, we observed a reduction in bursa lesion score at 14 days after challenge in high methionine fed birds in our previous study [6].

5. Conclusion

Although there was no interaction between methionine × fish oil for plasma fatty acid profile, the significant interaction of cytokine response showed that a balance of moderate level of fish oil (2.5%) and methionine level (twice NRC recommendation) might enhance immune response in IBD challenged broiler chickens.

Conflict of Interests

The authors declare that they have no conflict of interests.

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