Performance, Carcass Characteristics, Cardio-Pulmonary Morphometry, Gut-Morphology, Fatty Acid Profile and Humoral Immunity of Commercial Broiler Birds Fed Diet Supplemented with Blended Fish Protein

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Abstract

A 35-day trial was conducted to explore if different sources of dietary proteins at varying levels of metabolizable energy had any effect on the average daily feed intake (ADFI), average daily gain (ADG), feed efficiency (FE), carcass characteristics, cardio-pulmonary morphometry, gut morphology, profile of unsaturated fatty acid (UFA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and humoral immunity of the broiler birds. Total 288 Ross-308 day old male broiler birds were randomly distributed in four dietary treatment groups in a 2×2 factorial arrangement (Two different sources of proteins, i.e., plant and animal with two different levels of metabolizable energy, i.e., low and high). Each treatment had nine replicates containing eight birds per pen. Results indicated that, the supplementation of blended fish protein substantially increased ($p<0.001$) the final body weight and decreased ($p<0.001$) the ADFI at 1-14 d, 15-35 d and 1-35 d compared with plant protein. The ADG increased at 1-14 d ($p<0.001$), 15-35 d ($p<0.05$) and 1-35 d ($p<0.05$) and the FE improved ($p<0.001$) at 1-14 d, 15-35 d and 1-35 d. Similarly, the high energy diet significantly increased ($p<0.001$) the final body weight and decreased the ADFI at 1-14 d ($p<0.001$), 15-35 d ($p<0.05$) and 1-35 d ($p<0.05$) compared with low energy diet. The ADG increased ($p<0.001$) and the FE improved at 1-14 d, 15-35 d and 1-35 d. The animal protein increased the thigh weight ($p<0.01$), the neck weight ($p<0.05$) and the ratio ($p<0.001$) of $\sum \text{UFA}:\sum \text{SFA}$ and $\sum \text{PUFA}:\sum \text{MUFA}$ in the breast muscles of the broiler birds. The GLM identified no interaction effect ($p>0.05$) of energy and protein on the ADFI, ADG, FE, cardio-pulmonary morphometry and humoral immunity of the broiler birds against Newcastle and Infectious Bronchitis diseases. The RV:TV remained within standard range indicating no symptoms of cardiac dysfunctions. It was concluded that the fishmeal supplemented high energy diet improved weight gain, feed efficiency and meat quality of broiler by increasing $\sum \omega\text{-3}$ and $\sum \omega\text{-7}$ fatty acids as well as the ratio of $\sum \omega\text{-3}:\sum \omega\text{-6}$, $\sum \text{UFA}:\sum \text{SFA}$ and $\sum \text{PUFA}:\sum \text{MUFA}$ in the breast muscles of the broiler birds.

Introduction

Protein plays an important role in all the vital processes of the animal body (Beski et al., 2015). It helps synthesis of body tissues for development, maintenance, and remodeling of essential organs of the rapidly growing broiler birds (Mills et al., 2019). Commercial broiler chickens have very high dietary protein requirement (Gous, 2007). Hence, recognizable proof of the ideal protein sources for broilers is important for enhancing their performance. The vegetable and animal proteins are the two most unique sources of protein in poultry diet. Plant proteins are the most widely used protein sources for broiler chicken that are normally less expensive than animal proteins. However, there are many limitations to their incorporation in poultry diet due to content of anti-nutritional factors (ANFs). The most commonly used plant protein source in broiler diet is soybean meal (SBM). The SBM has the highest feeding value among all the plant protein sources in terms of protein content, digestibility and amino acid blends required to meet the nutrient demands for the birds of almost all species (Cromwell, 1999, 2008). Despite potential virtues, the SBM has a high alliance of ANFs, i.e., haemagglutinins, phytic acid, goitrogens, saponins, tannins, gossypols, oxalates, lectins, trypsin and amylase inhibitors which decline the nutritive worth and limit its incorporation in the diet of the growing broiler (Yasothai, 2016).

The animal proteins, on the other hand, offer an elevated array of essential amino acids, fat soluble vitamins, vitamin B₁₂, calcium, phosphorus, and sensible measures of different micro-minerals with very high degrees of accessibility and lack of major ANFs, i.e., trypsin inhibitor, gossypol, saponin, and phytic acid (Cho and Kim, 2011; Hossain et al., 2013). Feeding unique animal protein sources to the chicks, particularly at an earlier stage, may enhance early gut development, improved physiological parameter, increased muscle enlargement and insusceptibility of the birds to different infectious diseases. Thus, the virtues for supplementation of the immunologically dynamic blends of animal proteins are the primary focal point for the performance, gut wellbeing and immunity of the living birds.

Fish meal is a natural, balanced animal protein supplement that is high in protein and energy. High biological value (Sifri, 1995), contents of lysine, methionine, arginine, choline, biotin, vitamin A, D, E, K, calcium, phosphorus and vitamin B₁₂ (Scanes, 2010) make the feeding value (Pike, 1999; Kolacz et al., 2003; Takahashi et al., 2004; Leeson and Summers, 2009) of fishmeal unquestionable for broiler. Fishmeal has been considered as a potential feed ingredient to supplement deficiencies of essential amino acids in poultry rations (Sifri, 1995; Pike, 1999). Several researchers have come to the conclusion that fishmeal contains unidentified growth factors responsible for palatability of feedstuffs and improvement in performance of the broiler chicken (Barlow and Windsor, 1984; El Boushy and van der Poel, 2000). Despite many advantages, higher cost relative to plant proteins and presence of trimethylamine in fish meal responsible for fishy smell in broiler meat limit their inclusion in poultry diet. Net cost-benefit analysis associated with incorporation of blended fish proteins at varying dietary energy levels and its subsequent effects on performance, carcass characteristics, cardio-morphometry, gut-morphology, fatty acid profile and humoral immunity in broiler birds are scant. We therefore, aimed to focus light on these grey areas through this study.

Materials And Methods

Study design, animals and housing

The experiment was conducted in a two way 2² factorial arrangements (Two different sources of proteins, i.e., plant and animal with two levels of Metabolizable energy, e.g., low and high). Total 256 Ross-308 day old male broiler chicks were randomly distributed into eight dietary treatment groups with four replicates having 8 birds per pen (Table 1). The chicks were purchased from Nahar Agro Ltd., Chattogram, Bangladesh. All chicks were examined for male, grade A, uniform size without abnormalities. Floor space for each bird was 0.17 square feet in brooding box and 1 square feet in the cage. The birds were exposed to continuous lighting for the 1st three days followed by 1–4 hours of dark for the remaining 1–4 weeks. The chicks were brooded at a temperature of 95°F, 90°F, 85°F and 80°F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Room temperature and humidity were measured by using wall mounted indoor analog thermo-hygrometer. Before arrival of the chicks, the shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding boxes, rearing cages and pens were cleaned in the same manner. After cleaning and disinfection, the house was left empty one week for proper drying. After drying, all doors and windows were closed. The room was fumigated with single strength fumigant (Adding 40 ml formalin to 20 g KMnO₄ for 100 cubic feet area) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed regularly. Feeders and drinkers were cleaned and washed with Timsen® solution (0.3% v/v) daily. All birds were vaccinated against Newcastle and Gumboro disease with both the primary and booster doses. After each vaccination, lemon juice as a vitamin-C supplement was supplied through drinking water to overcome the effect of stress due to vaccination.

Table 1
Design of the experiment

Dietary treatments		No. of treatments	No. of replicates	No. of day old male birds
Protein	Energy			
0 (Plant protein)	1 (Low energy)	1	9	9 × 8 = 72
0 (Plant protein)	2 (High energy)	1	9	9 × 8 = 72
1 (Fish protein)	1 (Low energy)	1	9	9 × 8 = 72
1 (Fish protein)	2 (High energy)	1	9	9 × 8 = 72
Total		4	36	4 × 9 × 8 = 288

Table 2
Starter (1–14 d) and finisher (15–35 d) rations for the experimental broiler birds

Ingredients	Ration for the experimental birds ¹							
	Starter (1–14 d)				Finisher (15–35 d)			
	LEPP	HEPP	LEAP	HEAP	LEPP	HEPP	LEAP	HEAP
Maize	54.19	53.49	54.39	54.09	57.00	57.75	57.66	57.75
Rice polish	1.00	0.50	1.70	1.00	2.00	1.00	2.30	1.50
DDGS	0.30	1.00	0.50	0.90	0.30	0.30	0.30	0.30
Soybean oil	0.75	2.00	0.50	1.80	2.00	3.00	1.80	3.00
Soybean meal	40.45	40.20	36.00	36.00	35.33	35.30	31.00	31.00
Fish meal ²	-	-	3.50	3.50	-	-	3.50	3.50
Lime stone	1.60	1.20	1.70	1.10	1.60	1.10	1.60	1.30
Dicalcium phosphate	0.90	0.80	0.90	0.80	1.00	0.80	1.00	0.90
L-Lysine	0.15	0.15	0.15	0.15	0.13	0.12	0.15	0.12
DL-Methionine	0.20	0.20	0.20	0.20	0.18	0.17	0.18	0.17
Vitamin premix ³	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Feedzyme ⁴	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Common salt	0.20	0.20	0.20	0.20	0.20	0.20	0.25	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values (% or otherwise stated)								
ME (kcal/kg) ⁵	2909.35	2997.50	2903.53	3000.74	3015.12	3098.82	3014.05	3100.50
Crude protein	23.01	22.99	23.05	23.06	21.07	21.00	21.09	21.01
Crude fibre	3.61	3.61	3.57	3.48	3.44	3.43	3.37	3.28
Ether extract	3.30	3.30	4.52	3.48	4.71	4.64	5.51	4.81
Calcium	1.02	1.02	0.85	1.22	0.97	1.03	0.89	1.19
Phosphorus	0.70	0.70	0.68	0.74	0.71	0.71	0.67	0.74
Available phosphorus	0.40	0.40	0.38	0.45	0.43	0.42	0.38	0.46
Sodium	0.01	0.01	0.01	0.04	0.04	0.01	0.01	0.04
Potassium	1.01	1.01	1.00	0.93	0.92	0.90	0.88	0.81
Magnesium	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13

¹LEPP = Low energy plant protein, HEPP = High energy plant protein, LEAP = Low energy animal protein, HEAP = High energy animal protein;

³Per 2500 g contained: Beta-Carotene (Vitamin A) 12000000 IU, Cholecalciferol (Vit-D₃) 2400000 IU, Alpha-Tocopherol (Vit-E) 23 g, Menadione (Vit-K₃) 2 g, Thiamine (Vit-B₁) 2.5 g, Riboflavin (Vit-B₂) 5 g, Pyridoxine (Vit-B₆) 4 g, Nicotinic acid 40 g, Calcium-D-Pantothenete 12.5 g, Cobalamin (Vit-B₁₂) 12 mg, Folic acid 800 mg, Biotin (Vit-B₇) 100 mg, Cobalt 400 mg, Copper 10 g, Iron 60 g, Iodine 400 mg, Manganese 60 g, Zinc 50 g, Selenium 150 mg, DL-Mehionine 100 g, L-Lysine 60 g, Calcium 679.6 g;

⁴Per 100 g contained: Cellulase 20000 IU, Xylanase 200000 IU, Protease 20 IU, Amylase 40000 IU, Phytase 20 IU, Pectinase 1400 IU, Invertase 400 IU, Hemicellulose 500 IU, Lipase 20 IU, α-Galactosidase 100 IU;

⁵Metabolizable energy (kcal/kg);

⁶As per standard procedure (AOAC, 2019);

⁷Apparent gross energy (kcal/kg).

Ingredients	Ration for the experimental birds ¹							
	Starter (1–14 d)				Finisher (15–35 d)			
	LEPP	HEPP	LEAP	HEAP	LEPP	HEPP	LEAP	HEAP
Manganese (mg/kg)	82.68	82.68	81.63	83.16	81.75	82.68	80.59	82.35
Zinc (mg/kg)	90.25	90.25	87.58	95.81	92.05	93.55	88.06	96.90
Copper (mg/kg)	18.06	18.06	18.00	18.11	18.07	17.32	17.25	17.36

Ingredients	Ration for the experimental birds ¹							
	Starter (1–14 d)				Finisher (15–35 d)			
	LEPP	HEPP	LEAP	HEAP	LEPP	HEPP	LEAP	HEAP
Analyzed value⁶								
AGE ⁷ (kcal/kg)	3651.13	3507.65	3579.39	3543.52	3757.06	3609.3	3683.18	3646.24
Crude protein	22.55	22.76	22.36	22.14	20.44	20.79	20.25	20.59
Crude fibre	3.54	3.57	3.46	3.34	3.34	3.40	3.24	3.21
Ether extract	3.23	3.27	4.38	3.34	4.57	4.59	5.29	4.71
Calcium	1.00	1.01	0.87	1.17	0.94	1.02	0.86	1.17
Phosphorus	0.69	0.69	0.66	0.71	0.69	0.70	0.64	0.73
¹ LEPP = Low energy plant protein, HEPP = High energy plant protein, LEAP = Low energy animal protein, HEAP = High energy animal protein;								
³ Per 2500 g contained: Beta-Carotene (Vitamin A) 12000000 IU, Cholecalciferol (Vit-D ₃) 2400000 IU, Alpha-Tocopherol (Vit-E) 23 g, Menadione (Vit-K ₃) 2 g, Thiamine (Vit-B ₁) 2.5 g, Riboflavin (Vit-B ₂) 5 g, Pyridoxine (Vit-B ₆) 4 g, Nicotinic acid 40 g, Calcium-D-Pantothenete 12.5 g, Cobalamin (Vit-B ₁₂) 12 mg, Folic acid 800 mg, Biotin (Vit-B ₇) 100 mg, Cobalt 400 mg, Copper 10 g, Iron 60 g, Iodine 400 mg, Manganese 60 g, Zinc 50 g, Selenium 150 mg, DL-Methionine 100 g, L-Lysine 60 g, Calcium 679.6 g;								
⁴ Per 100 g contained: Cellulase 20000 IU, Xylanase 200000 IU, Protease 20 IU, Amylase 40000 IU, Phytase 20 IU, Pectinase 1400 IU, Invertase 400 IU, Hemicellulose 500 IU, Lipase 20 IU, α-Galactosidase 100 IU;								
⁵ Metabolizable energy (kcal/kg);								
⁶ As per standard procedure (AOAC, 2019);								
⁷ Apparent gross energy (kcal/kg).								

Table 3

Initial live weight (ILW, g/bird), final live weight (FLW, g/bird), average daily feed intake (ADFI, g/bird/d), average daily gain (ADG, g/bird/d) and feed efficiency (FE, ADFI/ADG) of the broiler birds fed diet supplemented with two different sources of dietary protein and two different levels of metabolizable energy

Treatment factors ¹	ILW (g/bird)	FLW (g/bird)	Performance parameter								
			ADFI (g/bird/d)			ADG (g/bird/d)			FE		
			1–14 d	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d
Protein											
0	40.91	1982.95	33.65	116.52	83.37	30.79	91.99	55.20	1.10	1.27	1.51
1	41.97	2424.87	32.42	103.26	74.92	32.63	93.93	56.36	1.00	1.10	1.39
Energy											
1	40.89	2153.03	33.97	111.97	80.77	30.57	90.57	54.34	1.12	1.24	1.49
2	42.00	2254.79	32.10	107.81	77.53	32.86	95.35	57.21	0.98	1.13	1.36
Protein × Energy											
Protein ₀ × Energy ₁	40.90	1925.58	34.52	118.45	84.88	29.50	89.31	53.59	1.17	1.32	1.58
Protein ₀ × Energy ₂	41.73	2040.32	32.78	114.58	81.86	32.08	94.67	56.80	1.02	1.21	1.44
Protein ₁ × Energy ₁	41.68	2380.47	33.41	105.49	76.65	31.63	91.83	55.10	1.06	1.15	1.39
Protein ₁ × Energy ₂	42.27	2469.26	31.42	101.04	73.19	33.63	96.02	57.61	0.94	1.05	1.37
SEM ²	0.80	40.02	0.23	1.63	1.04	0.32	0.70	0.42	0.02	0.02	0.02
Significance ³											
Protein	NS	***	***	***	***	***	*	*	***	***	***
Energy	NS	***	***	*	*	***	***	***	***	***	***
Protein × Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ Protein, 0 = Low, 1 = High, Energy, 1 = Low, 2 = High;											
² SEM = Standard error of the means;											
³ NS = Non-significant (p > 0.05), * = Significant (p < 0.05), *** = Significant (p < 0.001);											
^{a–b} Means bearing different superscripts in a column differ (p < 0.05).											

Experimental diets

Feed ingredients were purchased from Pahartali market, Chattogram, Bangladesh. During purchase, cleanliness and date of expiry was checked. Dry mash was provided to the birds throughout the whole experimental period. Four different types of rations were formulated. Each ration was two different types i.e., starter (0 to 14 days) and finisher (15 to 28 days). All rations were iso-caloric and iso-nitrogenous. Feed was prepared manually and supplied ad-libitum to the birds on round small feeder and waterer for 0–7 days. After 7th day, these feeders and waterers were replaced by medium linear feeders (2.21 ft X 0.25 ft) and round waterers. At 15th day, large linear feeder (3.5 ft X 0.38 ft) and round waterers (3 liter capacity) were provided for feeding and drinking of the birds.

Chemical analyses

From each treatment, 100 g of prepared mash feed was taken and preserved in an air tight bag to carry out the chemical analysis. Chemical analyses of the feeds were carried out in triplicate for dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extract (EE), total ash (TA), calcium and phosphorus (P) in the animal nutrition postgraduate laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram as per standard procedure (AOAC, 2019). Moisture was estimated by Hot air oven (SLN-115, Pol-Eko-Aparatus SPJ, Poland). CP was estimated by micro Kjeldhal apparatus (Kjeldhal digestion unit SBS800, Kjeldhal distillation unit D1000, FoodAlyt, Germany). CF was estimated by using Ankom Fiber Analyzer (Fiberbag System-6, Gerhardt, Germany). EE was estimated by using Soxtec (RS-

232, SER-148, Velp Scientifica, 155 Keyland Court, Bohemia, NY 11716 - US). TA was estimated by the muffle furnace (HYSC, Non Yong Scientific Equipment Company Ltd., 874-1 Wolgye 4-dong, Nowon-gu, Seoul, Korea). Gross energy (GE) of mixed diets was estimated by using the bomb calorimeter (Parr 6200 Calorimeter, Parr Instruments Co., USA).

Performance parameter

Mortality was recorded as occurred, while average daily feed intake (ADFI), average daily gain (ADG) and feed efficiency (FE) were recorded at fortnightly intervals. Carcass characteristics, hematological and biochemical parameters were recorded at 5th week. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total feeds supplied to the birds. The FE was calculated dividing feed intake by weight gain.

Carcass characteristics

At day 35, three birds from each replicate were randomly selected and killed by severing the jugular vein and carotid artery. Once a bird was adequately bled out, it was scalded and feather was removed. After defeathering, the birds were eviscerated and the head and feet were removed as per standard technique (Jones, 1984). During evisceration process, abdominal fat, lung, liver, kidney, spleen, gizzard and proventriculus were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.

Cardio-pulmonary morphometry

The heart was isolated from the carcass immediately after slaughter. The data of heart weight, right ventricular weight, left ventricular weight, right ventricular diameter, left ventricular diameter, right ventricular thickness and left ventricular thickness were measured thereafter by using slide caliper (Wiika Vernier Caliper, 150 mm, WA-VC1150) and digital screw gauge (Mitutoyo, Quickmini, Mitutoyo corporation, Japan). The diameter of left and right ventricles were measured perpendicular to the long axis, at the maximum measureable dimensions and mean values were calculated.

Gut morphology

At day-30, two birds from each of 36 replicates were randomly selected for slaughter. The gastrointestinal tract was removed and separated into the three intestinal segments, i.e., duodenum, jejunum and ileum. The length, weight and diameter of duodenum was taken distally from the gizzard to the end of the pancreatic loop, the jejunum was taken distally from the pancreatic loop to Meckel's diverticulum, and the ileum was taken from the Meckel's diverticulum to the ileo-caecal junction. All measurements were triplicate and averaged later on.

Fatty acid profile

Lipids were extracted from the breast muscles (Pectoralis major and pectoralis minor) of the slaughtered birds by using a modified method (Folch et al., 1957). Fatty acid methyl esters were extracted by the gas chromatography using KOH/methanol. The Gas Chromatograph (Nexis, GC-2030, Shimadzu, Japan) was equipped with a robotic auto sampler (AOC 6000, Shimadzu, Japan), a hydrogen flame ionization detector (FID) and a quartz capillary chromatographic column (SH-Rt-2560; 100 m × 0.25 mm × 0.20 µm; Temperature range – 20 to 250°C; Cat.# 227-36311-01; Serial # 4, USA). The FID was set at 270°C. Nitrogen was used as make up gas with a flow rate of 24.0 ml/min, FID H₂ flow 32 ml/min, FID air flow 200 ml/min and split ratio 1:10. The column was set at 240°C with a primary pressure of 534.9 kPa, flow rate 1.2 ml/min and linear velocity 22.5 cm/s constant for 60 min. The fatty acids were identified by comparing the area of the peaks of the sample with that of known standards (SigmaAldrich, St. Louis, USA).

Humoral immune response

All chicks were vaccinated against Newcastle Disease virus (NDV) and Infectious Bursal Disease (IBD) virus. Blood samples were collected 7 days after each immunization, kept till clotting, and then, centrifuged at 3,000 g for 15 min, and the sera samples were stored at -80°C until determination of antibody titers. Serum antibody titers were determined by means of the hemagglutination inhibition (HI) test and ELISA for NDV and IBD respectively using ELISA test kit according to manufacturer instructions listed in the ELISA Kit (Symbiotic-USA).

Statistical analysis

Data were compiled and tested for outliers and multicollinearity by inter quartile range test and variance inflation factors. Normality of the response variables were checked by using normal probability plot and equality of variances was tested by Shapiro Wilk test. Data were analyzed for generalized linear model by using Stata 14.1 SE (Stata Corp LP, College Station, Texas, USA) considering ILW, FLW, ADFI, ADG, FE, carcass characteristics, cardio-pulmonary morphometry, gut-morphology, fatty acid profile and humoral immunity parameter as the outcome variable and the sources of protein as well as the levels of metabolizable energy as the treatment factor. Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was accepted at $p < 0.05$ for the Fisher's F-tests. The following statistical model was used:

Y_{ijk}	=	$\mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$
Where,		
μ	=	The intercept of the regression model;
α_i	=	The fixed effect of the 'i th ' level of the factor 'α' (Sources of protein) on the value observed in Y_{ijk} (i = plant, animal);
β_j	=	The fixed effect of the 'j th ' level of the factor 'β' (Level of energy) on the value observed in Y_{ijk} (j = low, high);
$(\alpha\beta)_{ij}$	=	The interaction effect of the of the 'i th ' level of the factor 'α' and the 'j th ' level of the factor 'β';
Y_{ijk}	=	The observed effects of the variable under study at the 'i th ' level of the factor 'α' and the 'j th ' level of the factor 'β' for the 'k th ' repetition of measurements;
ε_{ijk}	=	The random sampling error due to 'i th ' level of the factor 'α' and 'j th ' level of the factor 'β' at the 'k th ' repetition of measurements.

Results

Performance

Supplementation of blended fish protein substantially increased ($p < 0.001$) the final body weight and decreased ($p < 0.001$) the ADFI at 1–14 d, 15–35 d and 1–35 d compared with plant protein. The ADG increased at 1–14 d ($p < 0.001$), 15–35 d ($p < 0.05$) and 1–35 d ($p < 0.05$) and the FE improved ($p < 0.001$) at 1–14 d, 15–35 d and 1–35 d. Similarly, the high energy diet significantly increased ($p < 0.001$) the final body weight and decreased the ADFI at 1–14 d ($p < 0.001$), 15–35 d ($p < 0.05$) and 1–35 d ($p < 0.05$) compared with low energy diet. The ADG increased ($p < 0.001$) and the FE improved at 1–14 d, 15–35 d, and 1–35 d. The sources of dietary protein and the levels of metabolizable energy had no interaction effect ($p > 0.05$) on the ADFI, ADG, and FE.

Carcass characteristics

Supplementation of animal protein increased the thigh weight ($p < 0.01$) and the neck weight ($p < 0.05$). The sources of dietary energy did not influence ($p < 0.05$) the carcass characteristics. The sources of dietary protein and the levels of metabolizable energy had no interaction effect ($p > 0.05$) on the carcass characteristics of the broiler birds (Table 4).

Table 4

Carcass characteristics of the broilers fed diet supplemented with two sources of dietary protein and two different levels of metabolizable energy slaughtered at 35th day

Treatment factors ¹	Relative weight of the carcass components as % live weight												
	DP ²	BRW ³	BKW ⁴	TW ⁵	DW ⁶	SW ⁷	HW ⁸	NW ⁹	WW ¹⁰	LW ¹¹	GW ¹²	PW ¹³	AFW ¹⁴
Protein													
0	64.5	21.7	13.2	10.0	9.1	4.5	2.4	2.4	7.2	2.1	0.5	2.8	1.0
1	66.7	20.7	14.5	11.0	10.2	5.1	2.5	2.5	7.5	2.3	0.5	3.0	1.1
Energy													
1	66.3	21.3	14.1	10.7	10.0	4.9	2.6	2.5	7.4	2.1	0.5	2.9	1.0
2	64.9	21.2	13.6	10.3	9.3	4.7	2.4	2.4	7.2	2.3	0.5	2.9	1.1
Protein × Energy													
Protein ₀ × Energy ₁	65.3	22.6	12.5	10.2	9.4	4.6	2.5	2.4	7.2	2.2	0.5	2.7	1.0
Protein ₀ × Energy ₂	63.7	20.9	13.9	9.9	8.8	4.4	2.3	2.3	7.1	2.1	0.4	2.9	1.0
Protein ₁ × Energy ₁	67.4	20.0	15.8	11.2	10.5	5.3	2.6	2.6	7.6	2.0	0.5	3.1	0.9
Protein ₁ × Energy ₂	66.0	21.5	13.2	10.8	9.9	5.0	2.4	2.5	7.4	2.6	0.5	2.9	1.2
SEM ¹⁵	0.92	0.50	0.64	0.18	0.25	0.13	0.06	0.04	0.13	0.12	0.02	0.11	0.09
Significance ¹⁶													
Protein	NS	NS	NS	**	NS	NS	NS	*	NS	NS	NS	NS	NS
Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;													
² DP=Dressing percentage, ³ BRW=Breast weight, ⁴ BKW=Back weight, ⁵ TW=Thigh weight, ⁶ DW=Drumstick weight, ⁷ SW=Shank weight (g), ⁸ HW = Head weight (g), ⁹ NW=Neck weight, ¹⁰ WW=Wing weight, ¹¹ LW=Liver weight, ¹² GW=Gizzard weight, ¹³ PW=Proventriculus weight, ¹⁴ AFW=Abdominal fat weight;													
¹⁵ SEM = Standard error of the means;													
¹⁶ NS = Non-significant (p > 0.05), * = Significant (p < 0.05), ** = Significant (p < 0.01).													

Cardio-pulmonary morphometry

The sources of dietary protein and the levels of metabolizable energy had no main or interaction effects (p > 0.05) on cardio-pulmonary morphometry of the experimental birds. The RV:TV remained within standard range (< 0.25) indicating no symptoms of cardiac dysfunctions (Table 5).

Table 5

Cardio-pulmonary morphometry of the broiler birds fed diet supplemented with two sources of dietary protein and two different levels of metabolizable energy slaughtered at 35th day

Treatment factors ¹	Cardio-pulmonary morphometry									
	HW ²	HWL ³	LW ⁴	LWL ⁵	RV ⁶	LV ⁷	RV: TV ⁸	RD ⁹	LD ¹⁰	RD: LD ¹¹
Protein										
0	9.69	0.50	12.6	0.64	0.54	1.82	0.23	0.28	1.08	0.21
1	8.99	0.49	10.6	0.57	0.51	1.79	0.22	0.28	1.08	0.21
Energy										
1	9.15	0.49	11.6	0.63	0.51	1.81	0.22	0.28	1.05	0.21
2	9.53	0.49	11.6	0.59	0.54	1.80	0.23	0.28	1.10	0.21
Protein × Energy										
Protein ₀ × Energy ₁	9.91	0.52	12.3	0.64	0.55	1.91	0.22	0.29	1.08	0.21
Protein ₀ × Energy ₂	9.47	0.48	13.0	0.64	0.52	1.73	0.24	0.28	1.08	0.20
Protein ₁ × Energy ₁	8.39	0.47	11.0	0.61	0.47	1.70	0.22	0.26	1.03	0.20
Protein ₁ × Energy ₂	9.59	0.51	10.3	0.54	0.55	1.88	0.23	0.29	1.13	0.21
SEM ¹²	0.27	0.01	1.05	0.05	0.02	0.05	0.01	0.01	0.03	0.00
Significance ¹³										
Protein	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;										
² HW = Heart weight (g), ³ HLW = Heart weight (% live weight), ⁴ LW = Lung weight (g), ⁵ LLW = Lung weight (% live weight), ⁶ RV = Right ventricular weight (g), ⁷ LV = Left ventricular weight (g), ⁸ RV: TV = Weight of right ventricle: total ventricle, ⁹ RD = Right ventricular diameter (mm), ¹⁰ LD = Left ventricular diameter (mm), ¹¹ RD: LD = Diameter of right ventricle: total ventricle;										
¹² SEM = Standard error of the means;										
¹³ NS = Non-significant (p > 0.05).										

Gut morphology

The sources of protein and the levels of dietary energy had no (p < 0.05) effect on gut morphology of the broiler birds (Table 6).

Table 6
Gut morphology of the broiler birds fed diet supplemented with two sources of dietary protein and two different levels of metabolizable energy slaughtered at 35th day

Treatment factors ¹	Gut morphology ²										
	PW	GW	Duodenum			Jejunum			Ileum		
L	W	D	L	W	D	L	W	D	L	W	D
Protein											
0	11.29	60.16	39.69	16.05	3.19	93.06	53.73	2.95	60.17	25.71	2.95
1	12.08	59.22	35.36	14.37	3.21	95.76	48.73	2.97	64.88	29.36	3.07
Energy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	11.88	59.03	36.16	14.65	2.90	91.31	51.34	2.84	57.04	23.60	2.87
2	11.09	57.15	39.69	16.78	3.16	91.35	53.26	3.03	69.38	32.62	3.18
Protein × Energy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Protein ₀ × Energy ₁	11.88	56.96	38.51	15.93	3.29	92.91	56.22	2.82	55.86	24.86	2.97
Protein ₀ × Energy ₂	11.88	60.35	35.28	14.28	3.18	97.47	45.07	2.37	51.74	17.20	2.62
Protein ₁ × Energy ₁	13.07	59.22	37.04	15.27	2.94	93.48	53.21	3.10	72.91	41.10	3.49
Protein ₁ × Energy ₂	11.29	58.09	33.75	13.55	3.53	96.33	47.91	3.44	69.97	29.80	3.08
SEM ³	12.47	50.20	37.63	12.69	2.35	87.21	46.01	2.82	72.32	30.64	3.20
Significance ⁴											
Protein	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein × Energy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¹ Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;											
² L = Length (cm), W = Width (mm), D = Diameter (mm);											
³ SEM = Standard error of the means;											
⁴ NS = Non-significant (p > 0.05).											

Table 7

Fatty acid profile of the breast meat (Pectoralis major) of the broiler birds fed diet supplemented with two sources of dietary protein and two different levels of metabolizable energy

Fatty acids	Protein		Energy		Protein × Energy ¹				SEM	Significance		
	0	1	1	2	Protein ₀ × Energy ₁	Protein ₀ × Energy ₂	Protein ₁ × Energy ₁	Protein ₁ × Energy ₂		Protein	Energy	Protein × Energy
C4:0	0.00	0.42	0.21	0.21	0.00	0.00	0.42	0.43	0.08	***	NS	NS
C10:0	0.00	4.40	2.23	2.17	0.00	0.00	4.46	4.33	0.83	***	NS	NS
C14:0	0.45	0.43	0.40	0.49	0.39	0.52	0.40	0.47	0.02	NS	***	***
C15:0	1.48	3.25	2.25	2.48	1.16	1.80	3.35	3.15	0.35	***	NS	***
C16:0	22.26	19.60	20.41	21.45	21.25	23.28	19.58	19.62	0.58	***	NS	***
C16:1, ω-7	1.95	3.09	3.34	1.69	3.52	0.38	3.16	3.01	0.47	***	***	***
C17:0	1.93	0.37	0.38	1.92	0.33	3.53	0.44	0.31	0.52	**	**	***
C17:1, c10, ω-7	0.57	1.22	0.93	0.87	0.53	0.62	1.33	1.12	0.13	***	***	***
C18:0	6.95	4.65	6.19	5.40	6.10	7.79	6.29	3.01	0.66	NS	***	***
C18:1, t9, ω-9	14.23	0.58	0.47	14.34	0.26	28.21	0.69	0.48	4.54	*	NS	***
C18:1, c9, ω-9	16.46	24.20	28.43	12.22	32.92	0.00	23.95	24.45	4.64	***	NS	***
18:2, c9,12, ω-6	27.21	26.34	25.76	27.80	26.26	28.17	25.26	27.43	0.49	NS	*	NS
18:3, c6,9,12, ω-6	0.27	0.74	0.59	0.42	0.54	0.00	0.65	0.84	0.12	***	***	***
C20:1, c11, ω-9	0.65	0.86	0.87	0.65	0.85	0.46	0.89	0.84	0.07	NS	NS	***
C18:3, c9,12,15, ω-3	1.29	2.04	1.43	1.89	1.60	0.97	1.27	2.82	0.27	***	***	***
C20:2, c11,14, ω-6	0.61	0.65	0.69	0.57	0.72	0.50	0.66	0.64	0.03	***	NS	***
C20:3, c8,11,14, ω-6	0.37	0.92	0.83	0.45	0.73	0.00	0.93	0.91	0.14	***	NS	***
C20:4, c5,8,11,14, ω-6	3.23	4.63	3.66	4.20	2.66	3.80	4.66	4.60	0.31	***	NS	***
C21:5, c5,8,11,14,17, ω-3	0.12	0.00	0.12	0.00	0.24	0.00	0.00	0.00	0.04	***	***	***
C24:1, c15, ω-9	0.00	1.16	0.59	0.57	0.00	0.00	1.17	1.15	0.22	***	*	NS
C22:6, c4,7,10,13,16,19, ω-3	0.00	0.48	0.26	0.23	0.00	0.00	0.51	0.46	0.09	***	***	**
Σω-3	1.40	2.52	1.81	2.12	1.84	0.97	1.78	3.27	0.31	*	***	***
Σω-6	31.68	33.27	31.52	33.43	30.89	32.46	32.14	34.40	0.57	NS	NS	NS
Σω-7	2.52	4.30	4.26	2.56	4.05	1.00	4.48	4.13	0.53	***	***	***

¹Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;

²ΣSFA = Sum of saturated fatty acids;

³ΣUFA = Sum of unsaturated fatty acids;

⁴ΣMUFA = Sum of mono-unsaturated fatty acids;

⁵ΣPUFA = Sum of poly-unsaturated fatty acids.

Fatty acids	Protein		Energy		Protein × Energy ¹				SEM	Significance		
	0	1	1	2	Protein ₀ ×	Protein ₀ ×	Protein ₁ ×	Protein ₁ ×		Protein	Energy	Protein ×
					Energy ₁	Energy ₂	Energy ₁	Energy ₂				Energy
∑ω-9	31.34	26.80	30.35	27.79	34.01	28.67	26.70	26.91	1.12	***	NS	***
∑ω-3:∑ω-6	0.04	0.08	0.06	0.06	0.06	0.03	0.06	0.10	0.00	***	NS	NS
∑SFA ²	33.07	33.10	32.06	34.11	29.22	36.91	34.91	31.30	1.15	***	***	***
∑UFA ³	66.94	66.90	67.94	65.89	70.79	63.09	65.10	68.70	1.15	***	***	***
∑MUFA ⁴	33.86	31.10	34.62	30.35	38.06	29.66	31.18	31.03	1.24	***	NS	***
∑PUFA ⁵	33.08	35.80	33.33	35.55	32.73	33.43	33.92	37.67	0.79	NS	**	NS
∑UFA:∑SFA	2.07	2.03	2.15	1.95	2.43	1.71	1.87	2.20	0.11	***	***	***
∑PUFA:∑MUFA	1.00	1.15	0.98	1.17	0.86	1.13	1.09	1.22	0.05	***	*	NS
¹ Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;												
² ∑SFA = Sum of saturated fatty acids;												
³ ∑UFA = Sum of unsaturated fatty acids;												
⁴ ∑MUFA = Sum of mono-unsaturated fatty acids;												
⁵ ∑PUFA = Sum of poly-unsaturated fatty acids.												

Fatty acid profile

Replacement of plant protein with fish protein substantially increased ($p < 0.001$) the relative proportions of butyric, decanoic, myristic, pentadecanoic, palmitoleic, cis-10 heptadecanoic, γ-linolenic, linolenic, cis-11,14 eicosadienoic, cis-5,8,11,14 eicosatrienoic, nervonic, docosahexaenoic, total ω-3, ω-7 and the ratio of ∑ω-3:∑ω-6, ∑UFA:∑SFA and ∑PUFA:∑MUFA in the breast muscles of the broiler birds.

Humoral immunity

The sources of dietary protein and the levels of metabolizable energy had no main or interaction effects ($p > 0.05$) on humoral immunity of the broiler birds against Newcastle and Infectious Bronchitis Diseases (Table 8).

Table 8

Immunity parameters of the broiler birds against Newcastle (ND) and Infectious Bronchitis Disease (IBD) supplemented with two sources of dietary protein and two different levels of metabolizable energy

Treatment factors ¹	Humoral immunity parameters			
	ND		IBD	
	ND HI titer at 7th d first vaccination	ND HI titer at 7th d booster vaccination	IBD titer at 7th d first vaccination	IBD titer at 7th d booster vaccination
Protein				
0	24.88	24.13	0.13	0.75
1	25.25	24.00	0.00	0.63
Energy				
1	25.00	24.00	0.13	0.50
2	25.13	24.13	0.00	0.88
Protein × Energy				
Protein ₀ × Energy ₁	24.75	24.00	0.25	0.50
Protein ₀ × Energy ₂	25.00	24.25	0.00	1.00
Protein ₁ × Energy ₁	25.25	24.00	0.00	0.50
Protein ₁ × Energy ₂	25.25	24.00	0.00	0.75
SEM ²	0.17	0.14	0.06	0.12
Significance ³				
Protein	NS	NS	NS	NS
Energy	NS	NS	NS	NS
Protein × Energy	NS	NS	NS	NS
¹ Protein 0 = Low, 1 = High, Energy 1 = Low, 2 = High;				
² SEM = Standard error of the means;				
³ NS = Non-significant (p > 0.05).				

Cost-benefit analysis

The high energy fish protein diets resulted maximum economic benefit compared with vegetable based low energy diets (Table 9).

Table 9

Cost-benefit analysis of the broiler birds fed diet supplemented with two different sources of dietary protein and two different levels of metabolizable energy

Line items	Dietary treatments ¹			
	LEPP	HEPP	LEAP	HEAP
Cost items (BDT ²)				
Final weight	1.93	2.04	2.38	2.47
Feed efficiency	1.58	1.44	1.39	1.37
Feed intake/bird	3.04	2.94	3.31	3.38
Feed cost/kg	35.98	37.87	40.75	41.64
Total feed cost/bird	109.47	111.26	134.84	140.86
Day old chick	65.00	65.00	65.00	65.00
Vaccination	5.00	5.00	5.00	5.00
Medication	5.00	5.00	5.00	5.00
Repair of house	5.00	5.00	5.00	5.00
Feeder/waterer	5.00	5.00	5.00	5.00
Incandescent light	5.00	5.00	5.00	5.00
Salary of labor/attendant	45.00	45.00	45.00	45.00
Depreciation of house/equipment	5.00	5.00	5.00	5.00
Miscellaneous ³	15.00	15.00	15.00	15.00
Total cost per broiler	262.97	266.26	289.84	295.86
Total cost per kg broiler	136.56	130.50	121.76	119.82
Return items				
Market price per kg live broiler	138.00	138.00	138.00	138.00
Market price per broiler	265.73	281.56	328.50	340.76
Net profit per live broiler	2.76	15.30	38.67	44.89
Net profit per kg live broiler	2.94	7.50	16.24	18.18
¹ LEPP = Low energy plant protein, HEPP = High energy plant protein, LEAP = Low energy animal protein, HEAP = High energy animal protein;				
¹ 1 USD\$ = 85.00 BDT;				
³ Electricity, transport, water, disinfectant, litter materials, cleaning and cooling devices.				

Discussions

Performance

In the present study, we observed decreased ($P < 0.001$) feed intake in the broiler birds due to supplementation of fish protein. The trend appears consistent with a previous study where benefits of the inclusion of fishmeal on feed intake were less evident during earlier stages but later on it was exhibited (Karimi, 2006). The reasoning could be the presence of biogenic amines (Barnes et al., 2001; Karimi, 2006), poor digestible protein content and objectionable odor of the fish meal (Halliwell and Chirico, 1993) which the chicks were unable to utilize at the earlier stages. The result, however, contradicts with the findings of Oduguwa et al. (2021) who reported significantly ($P \leq 0.05$) higher daily feed intake of the broiler birds supplied animal protein than those of the other treatments. In our study, we ensured normal appearance and smell of the fish meal when subjected to visual appraisal. The actual discrepancies, therefore, could be due to the variations of the levels of

fish meal used, metabolizable energy and protein contents of the diets although incorporation of even 16.6% of the sun dried fish offal to the diets of growing RIR chicken did not affect feed intake in a previous study (Tera et al., 2011).

We further observed increased daily gain while birds were offered diets supplemented with fish protein compared with plant protein. The improved palatability of the fish protein, well balanced amino acid profile, higher efficiency of utilization and improved feeding values of the diets supplemented with fish proteins might have contributed to the better weight gain of broiler during the starter and finisher period with the maximum benefit evident at 15–35 d (Memon et al., 2002; Metwally, 2004). It was also reported that this response was dose dependent although Karimi (2006) explained that the further increase of the levels of fish meal beyond optimum growth response was affected by sex and age of the birds (Negesse and Tera, 2010; Melesse et al., 2013). In another study, it was claimed that the fishmeal could be used at different levels up to 8% in broiler diets without adversely affecting weight gain (Awoniyi et al., 2003). Santana-Delgado et al. (2008) further demonstrated that there were no differences in the weight gain of the broiler chicken providing diet with either soybean meal or fishmeal. Similar results were reported elsewhere (Johnson et al., 1985).

We observed improved feed efficiency (FE) for the fish protein supplemented diets compared with the plant protein. The result is consistent with Hossain et al. (2012) who explained that the better protein digestibility of the fish protein fortified diets as well as increased efficiency of utilization of energy available in fish proteins were responsible for improved FE (Ali et al., 2001; Alali et al., 2011). This response, however, may not always be consistent with all the fish by-products used for feed formulation since the sources of protein may vary in property, i.e., the protein quality, digestibility, biological value, amino acid profile, physical and chemical properties (Sing and Panda, 1992). These characteristics of individual protein source may affect performance of the birds. However, several researchers reported that the major factor limiting the use of vegetable proteins in practical diet is the existence of naturally occurring deleterious factors including non-starch polysaccharides, tannins and trypsin inhibitors which have adverse effects on nutrient digestibility and absorption (Gatel, 1994; Smits and Annison, 1996). Saxena et al. (1962) reported that the unavailability of amino acids due to presence of anti-nutritional factors was considered a major factor in explaining the poor feed efficiency of the chicks fed diets containing soybean meal. Accordingly, despite being iso-caloric and iso-nitrogenous, better overall performance of the broiler birds in fish protein supplemented diets compared with soybean meal in our study is likely.

Carcass, cardio-pulmonary morphometry and gut morphology

We observed no changes in the carcass yield, breast meat yield, thigh yield, abdominal fat, liver weight, and heart weight for the sources of dietary protein and energy. It implies that the dietary sources of protein and energy did not alter the yield of carcass and the amount of its associated abdominal fat. Closely similar results were reported in the previous studies (Fanimu et al., 1996; Al-Marzooqi et al., 2010) where size of the liver, gizzard, heart and spleen did not differ significantly between the dietary treatments. The reasoning was that the adequate levels of essential amino acids in both the diets, particularly lysine and methionine used for protein accretion in body and a constant ME:CP ratio was maintained across all the dietary treatments (Si et al., 2001; Baker et al., 2002). Similarly, Hidalgo et al. (2004) also reported no differences in carcass yield, breast meat yield, and abdominal fat pad in broilers fed low CP diets with a constant ME:CP ratio. Dozier and Moran (2001) reported that feeding broiler birds with diets formulated to contain suboptimum levels of ME and CP impaired the amount and yield of carcass components. Contrastingly, Mikulec et al. (2003) reported that the addition of fish protein supplements enhanced the higher values for breast, thigh and drumstick.

Humoral immunity

In our study, we failed to elucidate the influence of the sources of protein and the levels of metabolizable energy on humoral immunity of the broiler birds. Fish meal contains plasma ceruloplasmin which is an indicator of acute phase inflammation in chicken associated with production or release of inflammatory cytokine (Chamanza et al., 1999). Thus, the results of the present study suggested that the dietary fish meal probably contained anti-inflammatory factors. The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of fish meal and fish oil can prevent cardiovascular diseases and altogether affect the vascular and haemo-static systems, the brain, retina and other body tissues (Stansby et al., 1965; Visentainer et al., 2000). Usually, materials of sea foods and fish meals contain greater amount of carnitine and taurine. Thus, it was hypothesized that the carnitine and taurine particularly rich in fish protein sources were concerned in the better immune performance observed in chicks fed diet containing fish meal during immune stimulation. Previous studies reported that the dietary carnitine increased the plasma IGF concentration (Kita et al., 2002) and immune response. Mast et al. (2000) suggested that the carnitine enhances the host-defense system. However, effects of dietary carnitine and taurine on broiler performances have not yet reached substantial agreement in other studies (Dunnington et al., 1996; Buyse et al., 2001; Zhan et al., 2006).

We deserved more studies to clarify the physiological nature of the dietary supplemented carnitine and taurine as factors stimulating growth and immune response in fish meal. It was hypothesized that a dietary protein source with higher biological value will result a lower urea and uric acid concentrations in blood serum compared to those with lower biological values (Bandegan et al., 2010). Dietary supplementation with more than 3.3 g EPA + DHA/100 kg BW daily (and in some studies up to 20.7 g EPA + DHA/100 kg BW) has been reported to decrease tumour

necrosis factor- α (TNF- α), interleukin (IL)-1, IL-2, IL-6 and interferon (IFN)- γ by mononuclear cell (Early, 1993). Accordingly, it was reported that the fish meal high in n-3 fatty acids, when fed to poultry, can increase the n-3 content in the bird's flesh, thus making this tissue another source of n-3 fatty acids in the human diets (Calder, 2001). Many researchers have demonstrated that the n-3 PUFA has anti-inflammatory properties that benefit the immune system, with particular effectiveness against asthma and rheumatic arthritis (Gerster, 1995; Calder, 2001). Since such direct effects against Newcastle and Infectious Bursal diseases are scant, therefore, similar humoral immune response from fish meal might be anticipated in our study based on ND and IBD titer values.

Fatty acid profile

It was reported that the fish meal high in n-3 fatty acids when fed to poultry increased the ω -3 fatty acid contents in the muscle of the broiler birds which made those tissues another source of ω -3 fatty acids for the human diets (Early, 1993). Ponnampalam et al. (2002) further reported that the long-chain ω -3 fatty acid contents (20:5n-3, 22:5n-3 and 22:6n-3) in the longissimus thoracis muscle were substantially higher with fish meal. Accordingly, the fish oils available in the fish meal not only increase the long chain ω -3 fatty acids in the meat, but also improve the ω -6: ω -3 ratio (Phetteplace and Watkins, 1989). In the present study we obtained substantially high levels of total ω -3 fatty acids and the ratio of $\sum \omega$ -3: $\sum \omega$ -6, $\sum$ UFA: $\sum$ SFA and $\sum$ PUFA: $\sum$ MUFA in the Pectoralis major muscle of the broiler birds fed diet supplemented with blended fish protein which are fully aligned with previous studies.

Conclusion

Fishmeal supplemented high energy diet improves weight gain, feed efficiency and meat quality of broiler by increasing $\sum \omega$ -3 and $\sum \omega$ -7 fatty acids as well as the ratio of $\sum \omega$ -3: $\sum \omega$ -6, $\sum$ UFA: $\sum$ SFA and $\sum$ PUFA: $\sum$ MUFA in the breast muscles of the broiler birds.

Declarations

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Conflicts of interest/Competing interests

None.

Availability of data and materials

All the data used in the manuscript exclusively belongs to the mentioned authors.

Code availability

None.

Author's contribution

Dr. Md. Emran Hossain conceived the study, procured research grant, led the research team, analyzed data, interpreted the results and finalized the draft. Dr. Nasima Akter conducted the animal trial, ran the immunization program, dressed the carcass and collected data. Dr. Sonnet Podder executed the cardio-pulmonary morphometry. Dr. Karabi Barua prepared the initial draft. Dr. Ahasanul Hoque provided additional insights. All authors read and approved the final manuscript.

Ethics approval

The entire experimental protocol was approved by the animal welfare law in Bangladesh (Memo No. CVASU/Dir(R&E)EC/2021/244(3/2).

Consent for publication

Yes.

Consent to participate

Yes.

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