Utilization of lactobacillus fermented proteins from meat processing wastewaters as a dietary protein source in poultry feed

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Research Article

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Abstract

Impact of fermented meat processing wastewater protein hydrolysate (FWMPH) prepared by *Pediococcus lolii* fermentation as an alternative protein source on growth performance, serum biochemistry, and hematological parameters of broiler chicks was evaluated in the present investigation. FWMPH exhibited antioxidant, anti-angiotensin converting enzyme (ACE), and antimicrobial properties. A total of 60 broiler chicks were divided into five dietary groups on day 8 and a feeding trial was performed for 48-days. Different diets were prepared by replacing soya protein concentrate (SPC) with different levels of FWMPH as a protein source. These diets were denoted as SPSD (100% SPC), FMP-25 (25% FWMPH + 75% SPC), FMP-50 (50% FWMPH + 50% SPC), FMP-75 (75% FWMPH + 25% SPC), and FMP-100 (100% FWMPH). FMP-25 and FMP-100 diet group broiler chicks showed the highest and lowest feed consumption ($P < 0.05$), respectively. However, the highest body weight gain (1506.08 ± 52.52 g) was observed in FMP-75 diet-fed chicks ($P < 0.05$). Furthermore, FMP-75 chicks displayed better feed conversion ratio, feed efficiency ratio, and protein efficiency ratio values than other diet group chicks ($P < 0.05$). The hematological, serum biochemical parameters and histopathological examination revealed that the chicks fed with FWMPH were not negatively affected by dietary treatments and were better than the SPSD diet group ($P < 0.05$). Hence, FWMPH at 75% level can be exploited as a potential protein ingredient as a substitute for soy protein in the broiler diet for better growth performance of the birds without deleterious effects.

Introduction

Poultry production has increased significantly over the last decades due to the growing population's high demand for animal proteins. In 2019, the global poultry meat production was 130 million tons and was expected to reach 137 million tons by 2020. India is one of the world's largest poultry meat producers, with more than 4 million tons in 2019 (FAO 2020). To meet the growing demand, the poultry sector in the country has grown at a rapid pace. Broiler chicks are the fastest growing and most efficient food species consumed globally among poultry birds. Broiler chicken provides tender meat with high-quality proteins for human consumption (Atteh 2004). Besides, broiler chicks require high dietary quality protein for good growth, immunity, adaptation to the environment. Protein is the second most significant ingredient in poultry diets. Therefore, protein source and their optimum level are crucial factors while formulating the broiler diets. Plant and animal origin proteins have been practiced in the poultry diets for many years. Mainly, soybean and fish meal are the most preferred protein sources used in the broiler diet due to their well-balanced amino acid composition (Iji et al. 2017; Teguia et al. 2002). However, these protein sources are expensive and limited, resulting in a hike in poultry feed production costs, which influences the profit margin of the poultry farmers. It has been ascertained that 70% of the total cost in poultry production drives to the feed cost.

Consequently, several cost-effective plant-based protein sources have been studied as alternative protein sources in the feed formulation for the growth performance of broiler chicks (Austic et al. 2013; Mishra et al. 2015; Tomaszewska et al. 2018). However, the main constraints of these protein sources are nutritionally unbalanced, deficient in certain essential amino acids, and contain anti-nutritional factors (Kermanshahi and Abbasi 2006; Talebali and Farzinpour 2005). On the other hand, animal proteins offer a high level of balanced amino acids in terms of essential amino acids (EAA). However, they are cost-intensive, limiting their use in commercial broiler production (Iji et al. 2017). Therefore, there is the need to search for alternative and cost-effective protein sources for poultry feed production. In this context, meat slaughterhouse wastewater proteins could be worthy of consideration.

Most of the wastewaters generated from local slaughterhouses and meat processing industries are legitimately released into nature with no appropriate treatment, fetching the calamitous environmental issues in India. Slaughterhouse wastewaters are known to be a good source of high-quality proteins. These proteins are rich in essential amino acids and exhibit several biological potentials (Bethi et al. 2020). Proteins generally enter the wastewaters primarily through visceral, flesh or tissues, and blood (Bethi et al. 2020). These wastewaters typically include surimi wash water, fish meat processing water, and blood water discharged directly into the water bodies without recovery of proteins leading to environmental problems (Bethi et al. 2020). Therefore, the recovery of valuable proteins from slaughterhouse wastewater streams would reduce the negative environmental impact and the cost of wastewater disposal and generate potential profit. Some reports have been archived that meat inferred proteins are healthfully better when looked at than those of plant sources (Iji et al. 2017; Teguia et al. 2002). Subsequently, the recovery of wastewaters proteins and their application in animal feed/food could upgrade the monetary and ecological advantages. A few techniques have been utilized to recuperate proteins from the wastewaters (Bethi et al. 2020).

Alkali solubilization and isoelectric precipitation is the most commonly used method for recovering protein from low-value biomass. In recent years, lactic acid bacteria (LAB) fermentation is emerging as a powerful tool for preparing protein hydrolysates. LAB fermentation improves the biomass's digestibility, quality, and physicochemical characteristics and provides probiotic substances' health benefits (Rai et al. 2011; Ruthu et al. 2014). The hydrolysis process generates amino acids and high-quality small or large peptides from the intact proteins, which strongly enhances its protein-functional and nutritional properties apart from its biological functions. Protein hydrolysate prepared by fermentative process offered better bio-functional properties such as antibacterial, antioxidative, antihypertensive, and immunomodulatory properties (Cian et al. 2011; Jemil et al. 2014; Korhonen and Pihlanto 2003). Moreover, fermentation is one of the best biotechnological preferable methods, chiefly in tropical countries due to the availability of favorable temperature, carbohydrate sources, and economically inexpensive. Recently, there has been gaining interest in the utilization of protein hydrolysates in animal diet due to its wide range of applications such as improvement in intestinal health, growth, and production performance. Researchers have reported on the application of protein hydrolysates from animal by-products for the growth performance in weaning

animals (Grazziotin et al. 2008; Oluba et al. 2019). In the same perspective, protein from meat processing wastewater steams could be a possible source of high-quality protein for the poultry feed formulation. Furthermore, LAB fermentation of meat processing wastewater protein could enhance the physicochemical and bio-functional properties apart from probiotic substances. Ironically, no study is found on the application of LAB fermented meat processing wastewaters proteins as functional protein ingredients for poultry's growth performance, especially in broiler chicken.

Against this background, producing protein hydrolysate from slaughterhouse waterwater and its use as animal feed could be economically and environmentally benefical. Therefore, the present study aimed to evaluate the *Pedicoccus lolii* fermented proteins as partial or full replacement of soy protein concentrate on growth performance, serum biochemistry, and histopathology.

Material And Methods

Chemicals and reagents

Newcastle Disease Vaccine (Live, B1 strain) and Infectious bursal disease vaccine (Live, B.P.) were obtained from Hester Bioscience Limited, India, and Indovax Private Limited, India. Day-old broiler chicks (VenCobb®), maize, and soy protein concentrate were procured from Karnataka Agro Vet Traders, India. Serum enzyme assay kits, glucose, albumin, cholesterol, creatinine, urea, and total protein were procured from Agappe diagnostic Ltd., Kerala, India. *Pedicoccus lolii* was collected from Institute Microbiology and Fermentation Technology Department, Council of Scientific and Industrial Research-Central Food Technology Research Institute, India. All other chemicals and reagents were of analytical grade and purchased from Merck, India.

Collection and protein recovery from meat processing wastewaters

Wastewaters were collected from fish, cattle, poultry, and goat slaughterhouses. Surimi processing wastewater was obtained during preparation of fish surimi. All the wastewaters were screened to remove grit and floating solids, thereafter subjected to protein recovery by alkali solubilization and isoelectric point precipitation method. The pH of each wastewater was adjusted to 11.0 by drop-wise addition of 6 M NaOH with stirring at 27 ^{o}C to solubilize the protein. Further, the pH of slaughterhouse wastewaters was brought down to 4.5 and surimi processing wastewater to 5.5 by adding 6 N HCl to accomplish the precipitation of solubilized wastewater proteins. The precipitate formed was stored at chilled (4 ^{o}C) condition for 2 h to settle down. The precipitated proteins of wastewaters were subsequently centrifuged (Thermo Scientific 2805R, US) at 4000 rpm, 4 ^{o}C for 20 min. Floating fat was removed manually and pellets were collected. These protein pellets were dissolved in demineralized water (1:0.20, w/v) and then neutralized (pH 7) using 2 M NaOH. Thereafter, equal volumes of each wastewater protein pellet were blended and named 'blended proteins' (BP) (Bethi et al. 2020). The blended protein was used for fermentative hydrolysis.

Protein hydrolysate preparation by P. lolii fermentation

Fermentative hydrolysis of the BP was performed using *P. lolii* employing optimized conditions; 18.4% BP, 1.8% carbohydrate, 10% inoculum, at 37 ^{o}C, 75 rpm for 56.18 h in 100 L reactor, keeping 60 L final volume. Obtained BP hydrolysate was spray-dried using 160 ^{o}C as inlet temperature and 130 ^{o}C as outlet temperature. Result powder was designated as lactobacillus fermented meat wastewaters protein hydrolysate (FWMPH).

Chemical analysis, antibacterial, and antioxidant potentials of FWMPH

The Association of Official Analytical Chemists technique was used to determine the moisture, protein, fat, and ash content of FWMPH (AOAC 2000). Antibacterial properties of FWMPH against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritis*, and *Staphylococcus aureus* were examined. The degree of hydrolysis (DH) of FWMPH was determined as described by Wisuthiphaet et al. (2016) using trinitro-benzene-sulfonic acid. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of FWMPH was performed according to Wu et al. (2003). Trolox equivalent antioxidant capacity (TEAC) of FWMPH was evaluated as per Raghavan et al. (2008). Ferric reducing antioxidant power (FRAP) of FWMPH was done as mentioned by Benzie and Strain (1996). Metal chelation and the ability of FWMPH to reduce ferric ions were determined by the method of Rocha et al. (2018). The iron-binding capacity of FWMPH was analyzed according to Decker (1990), Angiotensin-converting enzyme (ACE) inhibitory activity of FWMPH was performed as per Godinho et al. (2016).

Broiler chicken husbandry and diets formulation

The experimental design and procedures used in the present study were approved by Institute Animal Ethics Committee (IAEC No 118/2018) as per the guidelines of the Committee for Control and Supervision of Experiments on Animals, India. In total, 60 one-day-old straight-run broilers (VenCobb®) chicks were obtained from a commercial hatchery. All chicks were housed and fed a standard commercial diet from 1 to 7 days. On the 8th day, all chicks were weighed and wing tagged. Thereafter, nearly similar weight chicks were selected and disseminated in five dietary groups following a completely randomized design. Each dietary group had 12 chicks placed in five identical floor pens (1.5 x 1.5 m and stocking density of 12 chickens/m^2) deeply littered with rice husk.

The broiler feed for the starter, grower, and finisher phase was designed based on the protein content of FWMPH. Standard diet (SPSD) contained soya as a protein source. The FMP-25, FMP-50, FMP-75, and FMP-100 diets were formulated by replacing the soy protein with 25%, 50%, 75%, and

100% FWMPH, respectively. The experimental diet feeding period was divided into the starter phase and finisher phase. Chicks were fed with an experimental starter diet and finisher diet from day 8 to day 21 and day 22 to day 48, respectively. The proportion of every ingredient used to formulate the starter and finisher ratio is given in Table 1. Before feeding with experimental diets, chicks of each group were collectively weighed. Weighing the difference in the amount of diet supplied and consumed by each diet group was used to quantify the feed intake. Throughout the trial, daily feed consumption, protein intake, and weekly body weight gain of all diet groups chicks were evaluated. The chicks were vaccinated with Lasota (B1) on day 7 against Newcastle Disease and day 15 and day 22 against Gumboro disease IBD (infectious bursal disease). Vaccines were stabilized with skimmed milk powder (saline buffering) and administered through drinking water. The general health of chicks was monitored daily. Weighed quantities of feed and water were available for birds at all-time *ad libitum* throughout the experiment. On day 48, all broiler chickens were weighed and subjected to slaughtering and evisceration in the poultry slaughterhouse at the Institute facility.

Feed conversion (FCR), feed efficiency (FER), and protein efficiency (PER) ratio

FCR, FER, and PER were employed to assess the quality of the feed given to all diet groups of broiler chicks. The following equation was used to determine FCR (Tang et al. 2012):

$$\mathrm{FCR} = \frac{\text{Total feed intake}}{\text{Bodyweight gain}}$$

FER was determined by the following equation (Moreira et al. 2013):

$$\mathrm{FER} = \frac{\text{Bodyweight gain}}{\text{Total feed intake}}$$

PER was calculated by the following equation (Chisoro et al. 2018):

$$\mathrm{PER} = \frac{\text{Bodyweight gain}}{\text{Protein intake}}$$

Hematological and serum biochemistry analysis

Six chicken birds from each group fasted overnight before the slaughtering. Blood (5 ml) of fasted birds was collected by puncturing the jugular vein (jugular venepuncture) in a vial containing 1% ethylene diamine tetraacetic acid (EDTA). Collected blood samples were centrifuged, and the plasma was analysed for the hematological parameters and lymphocytes concentration using a hematology analyzer (Sysmex XP-100, India). Hematological examination such as white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin (MCHC) were performed.

Blood was collected without anticoagulant from the jugular vein of starved birds were allowed to stand for 1 hr in an ice bath for biochemical examination of serum. Furthermore, serum was isolated from blood by centrifuging at 3000 rpm for 15 mins at 4°C (Hettich rotina 420R, Germany). The separated serum was kept at -20°C until it was needed. Albumin, creatinine, cholesterol, total protein, urea, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) of serum samples were carried out using biochemical kits.

Liver biochemistry and antioxidant activities

Each bird group's removed liver was fixed in a 10% phosphate buffer saline solution. Liver tissue (100 mg) was homogenized in sucrose for 5 mins and the homogenate was centrifuged at 5000 rpm for 15 mins. The resulting supernatant was diluted in a 1:10 (v/v) ratio with 0.32 M sucrose before being analyzed. Standard kits were used to quantify cholesterol levels and the activity of essential marker enzymes such as SGPT, SGOT, and ALP (Agappe kits, India). Catalase (CAT) and superoxide dismutase (SOD) activity of the liver was determined according to Aebi (1984) and Marklund and Marklund (1974), respectively.

Relative organ weights and histopathology studies

The relative organ weight (ROW) of all diet group chicks was carried out considering the ratio of organ weight to body weight. ROW was presented in the g/100 g body weight of chicks. The slaughtered chick's vital organs, including the lungs, liver, heart, gizzard, and kidneys, were removed and weighed. Furthermore, all essential organs were removed, cleaned in saline, and was subjected to histological analysis. Tissues were fixed in paraffin and sliced into 5 m thick pieces in a uniform manner. The segments were stained with hematoxylin and eosin and compared to the SPSD chicks group microscopically to see any abnormalities.

Statistical analysis

All experiments were performed in triplicate. A completely randomized was used throughout the study. Data were expressed as means ± SD. Statgraphics software, version 16.1.11 was employed for data analysis. One-way analysis of variance was conducted, and means were compared by Duncan's multiple range test at a level of $P < 0.05$.

Results And Discussion

Chemical analysis, antibacterial, and antioxidant activities of FWMPH

In general, chemical compositions (protein, fat, ash, and moisture) of FWMPH in feed determines the energy requirement and growth performance of broiler chicks. FWMPH exhibited the 72.52 ± 1.61% protein content, suggests that FWMPH could be utilized as a protein supplement in broiler diets. FWMPH had the 13.43 ± 0.39% moisture, 7.67 ± 1.11% fat and 6.06 ± 0.63% ash content.

FWMPH demonstrated excellent antibacterial efficacy against *E. coli*, *S. aureus*, and *S. enteritidis*. Antibacterial potentials of FWMPH may improve the storage stability of feed and maintain healthier gut flora of broiler chicks. This can also be interpreted as preventing the spread of salmonella in feed, a food- and water-borne disease that is regularly seen in broilers. In addition to antibacterial activities, FWMPH exhibited the antioxidants activities, including, DPPH (531.31 ± 19.62 µmol Trolox equivalent/g of FWMPH), TEAC (382.49 ± 37.72 µmol Trolox equivalent/g of FWMPH), FRAP (404.76 ± 49.23 µmol Trolox equivalent /g of FWMPH), metal chelating activity (479.33 ± 81.69 µmol EDTA/g of FWMPH). FWMPH also displayed the iron-binding (76.81 ± 1.97%), and ACE-inhibition activity (35.17 ± 2.41%). Hence, FWMPH is the multi-biofunctional protein hydrolysate that could endorse biological and nutritional functions in the broiler chicken feed. It has been stated that the incorporation of multi-biofunctional protein hydrolysate as a protein ingredient in animal diet could improve gut health, feed efficiency, growth performance, and abilities to resist infectious diseases in animals (Hou et al. 2017). Therefore, FWMPH can be explored as biofunctional protein ingredient in the in poultry feed that could help in healthy growth performance of broiler chicks.

Daily feed intake (FI), protein intake (PI), and body weight gain (BWG) of broiler chicks

Figure 1 (a, b, and c, respectively) depicts daily FI, PI, and weekly BWG of broiler chicks fed with different diets (SPSD, FMP-25, FMP-50, FMP-75, and FMP-100). Protein is an important nutrient required for the growth and development of broiler chicks. Moreover, the protein quality influences the overall development and growth performance of broilers birds. In the present study, feed consumption (daily FI and PI) and BWG of all diet group chicks increased with the feeding period until the end of the feeding trial (6 weeks). The result demonstrates that the palatability and acceptability of the diet incorporated with FWMPH were better than the standard diet. Higher BWG by all group chicks might be due to higher digestibility of feed and a higher conversion rate into body mass. Further, maximum feed consumption by all diet groups was observed from the 3rd to the 6th week. Generally, during the growing phase (3rd week onwards), chicks required more feed energy for overall growth. Among the all-diet groups, the highest feed consumption showed by FMP-25 diet group chicks ($P < 0.05$), and the lowest consumption was presented by FMP-100 diet group chicks ($P < 0.05$). FWMPH at a higher level (100%) might contribute to the off-smell, off-taste, and off-color to feed, which resulted in reduced feed palatability and lower feed consumption by the FMP-100 diet group chicks. In general, chicken broilers are selective about feed characteristics like aroma, appearance, and taste. The sense of taste is essential to ensure adequate ingestion of nutrients and allow the broiler to select pleasant ingredients and discard those that are unpleasant (Cuevas et al. 2005). Further, SPSD, FMP-50, and FMP-75 showed no significant difference for feed consumption ($P > 0.05$). This result implied that the palatability of the FMP-50 and FMP-75 diets is similar to the SPSD diet. Therefore, the soy protein can be replaced with 50% or 75% FWMPH in the broiler diet. The average feed intake of SPSD, FMP-25, FMP-50, FMP-75, and FMP-100 was found to be 502.20, 528.70, 501.82, 492.60, and 455.70 g/chick, respectively.

The reliable indicator of growth is BWG as it sheds light on how the feed has influenced the development of broilers (Lovell 1989).

BWG is a good indicator for the determination of diet influence on growth of broiler chicks (Lovell 1989). In general, all FWMPH containing diet chicks exhibited higher body weight gain than SPSD group chicks throughout the feeding period. This might be because the FWMPH provides a high level of quality protein or essential amino acids than the soy protein (SPSD), resulting in higher body weight gain and development of chicks. It has been stated that meat protein is a high-quality protein due to a good balance of essential amino acids, higher digestibility, and biological value (Ravindran 2015; FAO 2013). Meat meals and fish meals have been used as high-quality animal protein sources in poultry diets (Oluba et al. 2019). Generally, broiler chicks require quality dietary protein for their better growth performance. The mean weekly body weight gain of SPSD, FMP-25, FMP-50, FMP-75, and FMP-100 during the 6 weeks was 640.44, 691.03, 747.11, 821.16, 736.14 g/chick, respectively. Among the FWMPH diet groups, FMP-75 presented the highest body weight gain compared to other FWMPH diet group counterparts ($P < 0.05$). The results indicated that a 75% FWMPH diet could help in gut development and rapid muscle growth at a higher extent and resulted in the highest weight gain of broiler chicks. On the other hand, FMP-25 diet group chicks showed the lowest body weight gain among the FWMPH diet groups though had higher feed consumption throughout the feeding study ($P < 0.05$). This might be associated with the lower content of FWMPH as a protein source in the diet when compared to other FWMPH diet counterparts. No significant difference in the body weight gain of chicks fed with FMP-50 and FMP-100 diets was noticed except in the 6th week ($P > 0.05$). Therefore, FWMPH at 75% level enhanced the bodyweight of the broiler chicks, compared to other diet groups.

FCR, FER, and PER

FCR, FER, and PER of broiler chicks fed with different diets are presented in Fig. 1d. Broiler chicks fed with the FMP-75 diet showed the lowest FCR (1.96) among the all diet group counterparts ($P < 0.05$). This result indicated the presence of a sufficient amount of good quality proteins in the FMP-75 diet, which resulted in the higher body weight gain of broiler chicks. Lower the FCR, higher the feed quality, and high weight gained by the broiler chicks. Besides, SPSD (2.57) and FMP-25 (2.55) diet groups exhibited the highest FCR values ($P < 0.05$), followed by FMP-50 (2.23) and FMP-100 (2.18). A higher FCR value represents low feed quality. Hence, SPSD and FMP-25 diets imply the low-quality feed among all the diet group counterparts. FCR values of SPSD and FMP-25 diet group and FMP-50 and FMP-100 diet presented no significant difference ($P > 0.05$). The FMP-75 broilers revealed the maximum FER (0.51) and PER (2.32) values, implying that a higher weight gain was obtained from the FMP-75 diet when compared to other diet groups. FMP-100 diet chicks had 0.46 FER and 2.09 PER values, followed by FMP-50 (0.45 and 2.04), FMP-25 (0.39 and 1.78), and SPSD (0.39 and 1.77), respectively. However, no significant difference was noticed for FER and PER values between FMP-100 and FMP-50 and between FMP-25 and SPSD diet groups ($P > 0.05$). Maliwan et al. (2017) also observed that Korat chickens had a higher feed intake but exhibited poor FCR, PER, and energy efficiency ratio values. Overall, FMP-75 diet had the potential to enhance the broiler's growth performance when compared to other diet counterparts. Therefore, 75% FWMPH could be used as a quality protein in broiler feed.

Hematology and serum biochemistry

The impacts of the different diets (SPSD, FMP-25, FMP-50, FMP-75, and FMP-100) on the hematological parameters of the broiler chicks are depicted in Table 2. All the experimental groups exhibited the hematology values within the normal reference range (Borsa et al. 2006; Cafe et al. 2012). The WBC, RBC, HGB, HCT, MCV, MCH, MCHC and LYM were in the range of 18.36–20.95 10^3/µL, 2.47–2.92 10^6/µL, 11.28–13.16 g/dL, 25.11–29.73%, 129.48-131.48 fL, 39.58–41.37 pg, 30.03–30.92 g/dL and 4.12–4.29%, respectively. FMP-75 fed broilers presented higher RBC (2.92 10^6/µL) and HGB (13.16 g/dL) values among all diet groups ($P < 0.05$). Accessible heam-iron in diet might have enhanced the RBC and HGB content. All FWMPH fed diet groups exhibited a significantly higher content of WBC, HCT, and MCH than SPSD ($P < 0.05$). Among the FWMPH fed groups, FMP-50 broilers displayed a slightly higher content of WBC. No significant difference in MCH values was noted among all FWMPH fed groups ($P > 0.05$). Furthermore, FMP-50 and FMP-75 diet groups exhibited the highest value of HCT compared to all diet groups. MCV was not significantly different across all diet groups. FMP-25 group showed slightly higher values of MCHC and LYM than others counterparts ($P < 0.05$). The lowest value of MCHC was noted in the SPSD diet group ($P < 0.05$), however, FMP-50, FMP-75, and FMP-100 diet groups did not show any significant difference in MCHC ($P > 0.05$). FMP-75 and FMP-100 diet groups presented the lowest content of LYM when compared to FMP-25, FMP-50, FMP-100 and SPSD diet group chicks ($P < 0.05$). In general, the comparable hematological parameters suggested that all group broilers chicks were healthy.

The serum biochemical profile of the broiler chicks is shown in Table 3. Generally, toxicity of feed mostly detected by the serum biochemical profile. Serum biochemical indices were influenced by different diet groups and are within the normal range established for broilers by Borsa et al. (2006) and Cafe et al. (2012). SGPT, SGOT, and ALP act as enzyme markers, and their levels in serum indicate the function of the liver. SGPT, SGOT, and ALP levels of all diet groups were in the range of 15.96–20.52 (U/L), 177.00-190.58 (U/L), and 167.50-179.01(U/L), respectively. FMP-100 diet group presented the slightly higher level of SGPT, SGOT, ALP, albumin, and cholesterol levels, followed by the FMP-75 diet groups ($P < 0.05$). The result revealed that the replacement of 100% soya protein by FWMPH in feed faintly boosted the levels of SGPT, SGOT, ALP, and cholesterol levels. Besides, lowest level of SGOT, ALP, and albumin were shown by the FMP-25 diet group ($P < 0.05$). However, SPSD and FMP-50 diet groups showed no significant albumin level difference ($P > 0.05$). Serum protein albumin synthesis gives the availability of protein and micro-nutrient in feed. SPSD diet group displayed the lowest SGPT and cholesterol levels ($P < 0.05$). The highest glucose level was found in the FMP-75 and FMP-100 groups, followed by the FMP-50 and FMP-25 diet groups ($P < 0.05$). No significant difference in glucose level between FMP-75 and FMP-100, and FMP-50 and FMP-25 was noted ($P > 0.05$). The highest creatinine level was noticed in FMP-50 to FMP-100 diet groups ($P < 0.05$), followed by FMP-25 and SPSD diet groups. No significant difference was noticed in triglycerides concentration in SPSD and FWMPH fed chicks ($P > 0.05$). FWMPH fed diet groups showed a slightly higher urea level compared to the SPSD diet group ($P < 0.05$). Conversely, the SPSD diet group presented a slightly higher level of total protein level when compared to FWMPH fed diet group counterparts ($P < 0.05$). Among the FWMPH fed groups, the FMP-100 showed a slightly higher protein value, followed by FMP-75 and FMP-50, respectively. Overall, it can be inferred from the hematological and serum biochemical parameters that the FWMPH did not have any adverse effects on the growth performance of broiler chicks. Therefore, 75% FWMPH can be used as a biofunctional protein ingredient in feed to maintain the nutrition status of broiler chicks.

Liver biochemistry

Liver biochemistry and antioxidant activities of broiler chicks fed with different diets are shown in Table 4. Liver is considered to be a model organ to find the toxicity of diet in broiler chicks. Despite this, the liver involves in the metabolism of fat, carbohydrate, protein, vitamins, and minerals. The liver of FMP-100 diet group chicks presented the highest level of SGPT, SGOT, and ALP, followed by FMP-75 and FMP-50 diet groups ($P < 0.05$). A similar trend for SGPT, SGOT, and ALP levels was noticed in the serum biochemical profile (Table 3). Slightly higher SGPT, SGOT, and ALP in the FMP-100 diet group may be due to a higher level of FWMPH. The FMP-25 diet had the lowest level of SGOT and ALP activity ($P < 0.05$), whereas, the SPSD diet group showed the lowest SGPT activity ($P < 0.05$). Furthermore, the liver of the FMP-75 and FMP-100 diet group exhibited the highest cholesterol level, followed by the FMP-50, FMP-25, and SPSD broilers ($P < 0.05$). The outcome demonstrated the presence of 75% FWMPH in the diet slightly enhanced the cholesterol levels. Conversely, SPSD and FMP-25 diet groups displayed the lowest content of triglycerides levels and highest found in the FMP-75 and FMP-100 diet groups ($P < 0.05$). Further, the highest total protein level was noted in the FMP-100 diet group, followed by the FMP-75 and FMP-50 diet groups, and lowest in the SPSD diet group ($P < 0.05$). The antioxidant capacity of FWMPH in broiler chicks was measured using SOD and CAT activities in the liver. FMP-75 diet group had the highest SOD and CAT activity, followed by FMP-100 and FMP-50, respectively.

Since FWMPH had an antioxidant potential, it was reflected in the *in vivo* broiler experiment and it can be concluded that a 75% FWMPH diet increased antioxidant capacity. However, as compared to the other diet groups, the SPSD diet group had the lowest SOD and CAT activities. Hence, substitution of 75% FWMPH in poultry feed could enhance the antioxidant potential in the broiler chick liver.

Relative organ weights and histopathology studies

The relative organ weights (g/100 g BW) of broiler chicks fed with different diets are shown in Table 5. The relative organ weights of broiler chicks also reveals the toxicity of feed. In the present study, FMP-75 and SPSD diet group chicks exhibited the highest and lowest relative organ weight of lungs ($P < 0.05$). The findings suggest that the FMP-75 diet may have aided proper or regular organ development and growth, owing to the inclusion of high-quality proteins (FWMPH) than the SPSD diet. The results were in line with the body weight gain of the broiler chicks (Fig. 1c). Further, no significant difference in relative organ weight of lung was noticed among the SPSD, FMP-25, and FMP-50 diet groups ($P > 0.05$). Tang et al. (2012) revealed that the organ weights of broilers were impacted by feeding the fermented ingredients. No significant difference in the liver weight of all diet groups was noticed ($P > 0.05$). Among all diet groups, FMP-75 showed the highest relative organ weight of heart, while, lowest presented by FMP-25 and FMP-100 diet groups ($P < 0.05$). Besides, FMP-25 and FMP-100 diet groups, and SPSD and FMP-50 diet groups revealed the no significant difference in heart organ weight ($P > 0.05$). The gizzard of SPSD and FMP-75 diet-fed broiler chicks exhibited higher relative weight in comparison to other groups ($P < 0.05$), however, no significant difference was found between them ($P > 0.05$). FMP-100 showed the lowest relative organ weight for kidneys, while other groups dint not have a significant difference ($P > 0.05$). In general, slight variations in relative organ weights all diet groups of chicks was observed but no adverse effect on the growth of lungs, liver, heart, gizzard, and kidney in broiler chicks. Overall, the FMP-75 diet group showed better growth of all studied organs compared to other diet group counterparts. Therefore, the FMP-75 diet could be feasible for the better growth and health of vital organs of broiler chicks.

The histological structure of the lungs, liver, heart, intestine, gizzard, and kidney are presented in Fig. 2. Diet can impact the structural characteristics of internal organs. No significant structural deviations in lungs, liver, heart, intestine, gizzard, and kidney of diet groups fed with FWMPH were noted. The result suggested that incorporation of FWMPH in broiler diets at all concentrations showed no significant toxic effects on vital organs of broiler chicks. Moreover, FWMPH and SPSD diet groups displayed maximum resemblances in the histological investigation of vital organs. The alveoli and bronchioles grew normally in all diet groups, indicating that there were no notable alterations in lung anatomy. In all diet groups, the histological structure of the liver exhibited normal hepatocytes, integrated architecture, portal veins, and no obvious symptoms of edema or hyperemia. Liver histopathology is well recognized examination for the investigation of feed toxicity in animal. All diet groups showed normal liver parenchyma structure with maintained portal and triad architecture. Across the all diet groups, the kidney revealed typical tubular structure nephrocytes and bowmen's capsules, with no noticeable alterations. The heart displayed striated muscle fibers with no gangrene or degeneration in all diet groups. The gizzard muscle of SPSD, FMP-25, FMP-50, and FMP-100 diet group chicks were striated, completely homogeneously stained, and showed normal architecture. However, the gizzard of the FMP-100 diet group showed the thickened lining with prominent folds and roughened, showing a bark-like appearance. Itakura (1981) reported that young chicks fed with the fish meal had mild lesions, which are natural. Those lesions had no relation to the fish meal consumed. The structure of villi showed minor ruptures across all the diet groups, but it is not influenced by the diet treatment. Overall, the result ascertained that incorporation of FWMPH in broiler chick's diet had no organ toxicity. Thus, the relative organ weight and the histopathological studies demonstrated that the addition of FWMPH up to 75% as a protein source in the broiler diet did not affect the normal growth of vital organs and did not have any toxicology effect on the growth performance of experimental broiler chicks. Thus, 75% of FWMPH could be a substitute in the standard broiler feed as an alternative dietary protein source.

Conclusion

Different meat processing wastewater streams could be the good source of valuable proteins.

Biofunctional (antioxidants, ACE-inhibition, and antibacterial) protein hydrolysates (FWMPH) can be prepared using lactobacillus fermentative approach from the proteins recovered from meat processing wastewaters. Substitution of biofunctional FWMPH as a protein source at 75% level in standard poultry feed enhanced the growth performance of broiler chicks without toxicological and pathological impacts. Moreover, the inclusion of 75% FWMPH in the diet enhanced the antioxidant enzymes in the broiler chicks, resulting in alleviating the harmful effects of oxidative stresses in broilers under challenging conditions. However, 100% FWMPH substitution in poultry feed led to the poor growth performance of broiler chicks. Hence, 75% FWMPH can serve as a protein substitute for the soybean protein concentrate in the broiler diet for better growth performance of the birds without any deleterious effects. Additionally, recovery and preparation of protein hydrolysate from meat processing wastewater streams through a fermentative approach would help in paving the way for a cleaner environment.

Declarations

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Ethical approval

The experimental design and procedures used in the present study were approved by Institute Animal Ethics Committee (IAEC No 118/2018) according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Consent to Participate

Patient or study participant not involved in this study.

Consent to Publish

The authors have consent for all information about this manuscript to be published in the Journal of Environmental Science and Pollution Research.

Authors Contributions

Cathrine M. S. Bethi: Involved in conducting the experiments, collection, and analysis of data and manuscript writing.

Gowthami Jayprakash: Involved in conducting the experiments and data analysis.

Muthukumar S. Pedda: Overall support in conducting broiler chicks feeding experiments.

Tanaji G. Kudre: Involved in conception, design, and implementation of the research, analysis of data, manuscript writing, and overall supervision of the project. All authors discussed the results and contributed to the final manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Aebi K (1984) catalase *in vitro*. Methods Enzymol 105:121–126
2. AOAC (2000) Official methods of analysis of AOAC International (17th ed.), Gaithersburg, MD, USA
3. Atteh JO (2004) Theory and practice of poultry production. Adlek printers. 64 Sabo-line, Ilorin, Kwara State, Nigeria. 2–4, 112–117
4. Austic RE, Mustafa A, Jung B, Gatrell S, Lei XG (2013) Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens. J Agric Food Chem 61:7341–7348
5. Bethi CMS, Narayan B, Martin A, Kudre TG (2020) Recovery, physicochemical and functional characteristics of proteins from different meat processing wastewater streams. Environ Sci Pollut Res 27:25119–25131
6. Benzie IFF, John JS (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 239:70–76
7. Borsa A, Kohayagawa A, Boretti LP, Saito ME, Kuibida K (2006) Serum levels of hepatic enzyme function in clinically healthy broiler chickens. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 58:675–677
8. Cafe MB, Rinaldi FP, Morais HR, Nascimento MRBM, Mundim AV, Marchini CFP (2012) Biochemical blood parameters of broilers at different ages under thermoneutral environment.World's Poult Sci J143–146
9. Chisoro P, Nkukwana TT, Mupangwa JF, Mabusela TP (2018) Feed intake, growth performance, and carcass traits of broilers fed diets with various inclusion levels of baobab seed oilcake. S Afr J Anim Sci 48:284–294
10. Cian RE, Luggren P, Drago SR (2011) Effect of extrusion process on antioxidant and ACE inhibition properties from bovine hemoglobin concentrate hydrolysates incorporated into expanded maize products. Int J Food Sci Nutr 62:774–780
11. Cuevas AC, Vega JLL, Gonzalez EA (2005) Influence of an appetite stimulant on feed consumption and productive performance on broiler chickens. Vet Mex 36:127–133
12. Da Rocha M, Aleman A, Baccan GC, Lopez-Caballero ME, Gomez-Guillen C, Montero P, Prentice C (2018) Anti-inflammatory, antioxidant, and antimicrobial effects of underutilized fish protein hydrolysate. J Aqu Food Product Tech 27:592–608

13. Decker EA, Welch B (1990) Role of ferritin as a lipid oxidation catalyst in muscle food J.Agr Food Chem38,674–677
14. FAO (2013) Food and Agriculture Organisation of the United Nations
15. FAO (2020) Food and Agriculture Organisation of the United Nations. Available online: http://www.fao.org/3/cb1329en/online/cb1329en.html#figure_26_tab
16. Grazziotin A, Pimentel FA, Jong EVDE (2008) Poultry feather hydrolysate as a protein source for growing rats. Braz J Vet Res Anim Sci 45:61–67
17. Godinho IC, Pires S, Pedro B, Teixeira R, Mendes ML, Nunes, Batista I (2016) Antioxidant properties of fish protein hydrolysates prepared from cod protein hydrolysate by *Bacillus* sp. Appl Biochem Biotechnol 178:1095–1112
18. Hou Y, Wu Z, Dai Z, Wang G, Wu G (2017) Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J Anim Sci Biotechnol 8:1–13
19. Iji PA, Toghyani M, Ahiwe EU, Omede AA (2017) Alternative sources of protein for poultry nutrition. Achiev sustainable Prod Poult meat 2:237–269
20. Jemil I, Jridi M, Nasri R, Ktari N, Ben R, Salem S, Mehiri M, Hajji M, Nasri M (2014) Functional, antioxidant, and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis*. Process Biochem 49:963–972
21. Kermanshahi H, Abbasi Pour AR (2006) Replacement value of soybean meal with rapeseed meal supplemented with or without a dietary NSP-degrading enzyme on performance, carcass traits, and thyroid hormones of broiler chickens. Int J Poult Sci 5:932–937
22. Korhonen H, Pihlanto A (2003) Food-derived Bioactive Peptides – Opportunities for Designing Future Foods. Curr Pharm Des 9:1297–1308
23. Lovell RT (1991) Nutrition of aquaculture species. J Ani Sci 69:4193–4200
24. Maliwan P, Khempaka S, Molee W (2017) Evaluation of various feeding programmes on growth performance, carcass, and meat qualities of Thai indigenous crossbred chickens. S Afr J Anim Sci 47:16–25
25. Marklund S, Marklund G (1974) Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur J Biochem 47:469–474
26. Mishra A, Ray S, Sarkar S, Haldar S (2015) Cotton Seed Meal as a Partial Replacement for Soybean Meal in Cobb 400 Broiler Rations. Indian J Anim Nutr 32:69–74
27. Moreira LM, Behling S, Rodrigues S (2013) Spirulina as a Protein Source in the Nutritional Recovery of Wistar Rats. Braz Arch Biol Technol 56:447–456
28. Oluba OM, Aran O, Akpor O (2019) Growth Performance and Toxicological Assessments of Chicken Feather Protein Hydrolysate as Fish Meal Substitute in Rat Diet. Asian J Sci Res 12:450–461
29. Rai AK, Jini R, Swapna HC, Sachindra NM, Bhaskar N, Baskaran V (2011) Application of native lactic acid bacteria (LAB) for fermentative recovery of lipids and proteins from fish processing wastes: Bioactivities of fermentation products. J Aquat Food Prod Technol 20:32–44
30. Raghavan S, Kristinsson HG, Leeuwenburgh C (2008) Radical scavenging and reducing ability of tilapia (*Oreochromis niloticus*) protein hydrolysates. J Agric Food Chem 56:10359–10367
31. Ravindran V (2015) Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. World's Poult Sci J 48:205–231
32. Ruthu PS, Rai AK, Bhaskar N (2014) Fermentative recovery of lipids and proteins from freshwater fish head waste with reference to antimicrobial and antioxidant properties of protein hydrolysate. J Food Sci Technol 51:1884–1892
33. Talebali H, Farzinpour A (2005) Effect of different levels of full-fat canola seed as a replacement for soybean meal on the performance of broiler chickens. Int J Poult Sci 4:982–985
34. Tang JW, Sun H, Yao XH, Wu YF, Wang X, Feng J (2012) Effects of Replacement of Soybean Meal by Fermented Cottonseed Meal on Growth Performance, Serum Biochemical Parameters and Immune Function of Yellow-feathered Broilers. Asian-australas J Anim Sci 25:393–400
35. Teguia A, Mpoame M, Okourou Mba J (2002) The Production Performance of Broiler Birds as Affected by the Replacement of Fish Meal by Maggot Meal in the Starter and Finisher Diets. Tropicultura 20:187–192
36. Tomaszewska E, Muszynski S, Dobrowolski P, Kwiecien M, Klebaniuk R, Szymanczyk S, Tomczyk A, Kowalik S, Milczarek A, Swietlicka I (2018) The influence of dietary replacement of soybean meal with high-tannin faba beans on gut-bone axis and metabolic response in broiler chickens. Ann Anim Sci 18:801–824
37. Wisuthiphaet N, Sopa K, Kongruang S (2016) Fish protein hydrolysate production by acid and enzymatic hydrolysis. J Appl Sci and Eng 9:4–8
38. Wu H, Chen H, Shiau C (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int 36:949–957

Tables

Table 1 Composition and calculated analysis of broiler starter and finisher diets fed to the broiler chicks.

Ingredients	Broiler starter diet (g/kg)	Broiler finisher diet (g/kg)
Maize	620.00	680.00
Soya protein concentrate	380.00	320.00
Calculated analysis (g/kg)		
Moisture	95.00	95.00
Crude protein	226.00	205.00
Crude fiber	18.40	20.10
Ash	100.00	100.00

Table 2 Hematological profile of the broiler chicks fed with SPSD and FWMPH at different levels (%).

Parameters	SPSD	FMP-25	FMP-50	FMP-75	FMP-100
WBC (10^3/µL)	18.36±0.64^{c}	19.59±0.52^{bc}	20.95±1.10^{a}	20.26±1.66^{ab}	19.45±0.86^{bc}
RBC (10^6/µL)	2.83±0.14^{ab}	2.47±0.10^{d}	2.66±0.14^{c}	2.92±0.06^{a}	2.73±0.09^{bc}
HGB (g/dL)	12.59±0.05^{b}	11.28±0.04^{c}	12.94±0.57^{ab}	13.16±0.51^{a}	12.64±0.23^{b}
HCT (%)	25.11±2.56^{c}	26.25±1.71^{bc}	28.82±0.81^{a}	29.73±0.96^{a}	27.74±1.12^{ab}
MCV (fL)	130.52±2.25^{a}	129.48±1.68^{a}	131.48±0.84^{a}	129.78±0.94^{a}	131.19±0.73^{a}
MCH (pg)	39.58±1.01^{b}	40.68±0.13^{a}	41.21±0.81^{a}	41.13±0.82^{a}	41.37±0.67^{a}
MCHC (g/dL)	30.03±0.52^{b}	30.92±0.70^{a}	30.18±0.59^{ab}	30.61±0.86^{ab}	30.86±0.58^{ab}
LYM (%)	4.22±0.12^{ab}	4.29±0.06^{a}	4.19±0.07^{ab}	4.12±0.11^{b}	4.13±0.13^{b}

Values are mean ± SD (n=6). Mean values within the same row with different lowercase superscripts are significantly different (P<0.05) according to Duncan's multiple range test. SPSD: standard (soya protein concentrate); FMP-25: 25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH; WBC: white blood cells; RBC: red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular hemoglobin concentration; LYM: Lymphocytes.

Table 3 Serum biochemistry profile of broiler chicks fed with SPSD and FWMPH at different levels.

Parameters	SPSD	FMP-25	FMP-50	FMP-75	FMP-100
SGPT (U/L)	15.96±0.68^{c}	19.03±0.57^{b}	19.42±1.03^{b}	19.84±0.63^{ab}	20.52±0.13^{a}
SGOT (U/L)	186.25±1.27^{b}	177.02±2.21^{d}	183.88±1.04^{c}	187.88±1.85^{b}	190.58±0.71^{a}
ALP (U/L)	167.50±1.07^{d}	165.89±0.78^{e}	172.79±0.61^{c}	175.30±0.53^{b}	179.01±0.53^{a}
Glucose (mg/dL)	111.27±0.83^{c}	124.83±0.77^{b}	125.71±0.55^{b}	128.64±0.49^{a}	128.06±0.76^{a}
Albumin (g/dL)	1.32±0.02^{c}	1.23±0.02^{d}	1.35±0.02^{c}	1.60±0.06^{b}	1.83±0.01^{a}
Cholesterol (mg/dL)	128.08±1.16^{e}	132.05±1.33^{d}	134.05±0.76^{c}	135.82±0.46^{b}	137.51±1.35^{a}
Creatinine (mg/dL)	0.38±0.01^{b}	0.39±0.01^{b}	0.41±0.01^{a}	0.42±0.01^{a}	0.42±0.01^{a}
Triglycerides (mg/dL)	132.39±0.64^{a}	131.68±0.52^{a}	131.58±0.91^{a}	132.20±0.91^{a}	131.87±0.51^{a}
Urea (mg/dL)	15.18±0.66^{b}	15.70±0.93^{ab}	15.39±0.99^{ab}	16.56±0.82^{a}	16.27±1.69^{ab}
Total protein (g/dL)	4.31±0.18^{d}	4.15±0.24^{d}	4.66±0.17^{c}	4.90±0.11^{b}	5.55±0.08^{a}

Values are mean ± SD (n=6). Mean values within the same row with different lowercase superscripts are significantly different (P<0.05) according to Duncan's multiple range test. SPSD: standard (soya protein concentrate); FMP-25:25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH; SGPT: Serum glutamic pyruvic transaminase; SGOT: serum glutamate oxaloacetate transaminase; ALP: Alkaline phosphatase; SOD: superoxide dismutase.

Table 4 Liver biochemistry analysis and antioxidant activity of the broiler chicks fed with SPSD and FWMPH at different levels (%).

Groups	Biochemical parameters						Antioxidant activities	
	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Cholesterol (mg/dL)	Triglycerides (mg/dl)	Total protein (mg/dl)	Catalase (U)	SOD (U)
SPSD	47.88±2.05^{c}	93.12±0.64^{b}	47.86±0.31^{d}	35.26±0.97^{b}	3.93±0.06^{a}	19.04±0.59^{d}	34.33±0.68^{d}	35.24±1.25^{d}
FMP-25	57.10±1.70^{b}	88.51±1.11^{d}	47.40±0.22^{e}	35.32±1.00^{b}	3.93±0.05^{a}	20.01±0.96^{c}	35.87±0.45^{c}	37.40±0.76^{c}
FMP-50	58.25±3.10^{b}	91.94±0.52^{c}	49.37±0.17^{c}	36.11±0.69^{b}	3.75±0.17^{b}	21.59±0.28^{b}	36.36±0.68^{bc}	42.45±0.96^{b}
FMP-75	59.53±1.90^{ab}	93.94±0.93^{b}	50.08±0.15^{b}	37.55±0.51^{a}	3.57±0.07^{c}	22.21±0.05^{b}	38.46±0.61^{a}	45.73±1.21^{a}
FMP-100	61.56±0.40^{a}	95.29±0.36^{a}	51.15±0.15^{a}	37.97±0.62^{a}	3.56±0.08^{c}	23.99±0.49^{a}	37.21±0.27^{b}	43.93±1.50^{ab}

Values are mean ± SD (n = 6). Mean values within the same row with different lowercase superscripts are significantly different (P<0.05) according to Duncan's multiple range test. SPSD: standard (soya protein concentrate); FMP-25: 25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH. SGPT: Serum glutamic pyruvic transaminase; SGOT: serum glutamate oxaloacetate transaminase; ALP: Alkaline phosphatase; SOD: superoxide dismutase.

Table 5 Relative organ weight of broiler chicks fed with SPSD and FWMPH at different levels (%).

Organ weight (g/100g BW)	SPSD	FMP-25	FMP-50	FMP-75	FMP-100
Lungs	0.30±0.15^{b}	0.37±0.20^{ab}	0.49±0.24^{ab}	0.57±0.17^{a}	0.37±0.18^{ab}
Liver	2.09±0.82^{a}	1.71±0.83^{a}	1.73±0.90^{a}	2.19±0.60^{a}	1.60±0.80^{a}
Heart	0.62±0.33^{ab}	0.54±0.25^{b}	0.56±0.27^{ab}	0.77±0.13^{a}	0.54±0.25^{b}
Gizzard	5.14±0.85^{a}	4.00±0.1.99^{ab}	3.64±1.71^{b}	5.07±1.94^{a}	3.88±1.86^{ab}
Kidneys	0.14±0.06^{a}	0.13±0.08^{a}	0.14±0.07^{a}	0.12±0.06^{a}	0.08±0.05^{b}

Values are mean ± SD (n = 12). Mean values within the same row with different lowercase superscripts are significantly different (P<0.05) according to Duncan's multiple range test. SPSD: standard (soya protein concentrate); FMP-25: 25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH; BW: body weight.

Figures

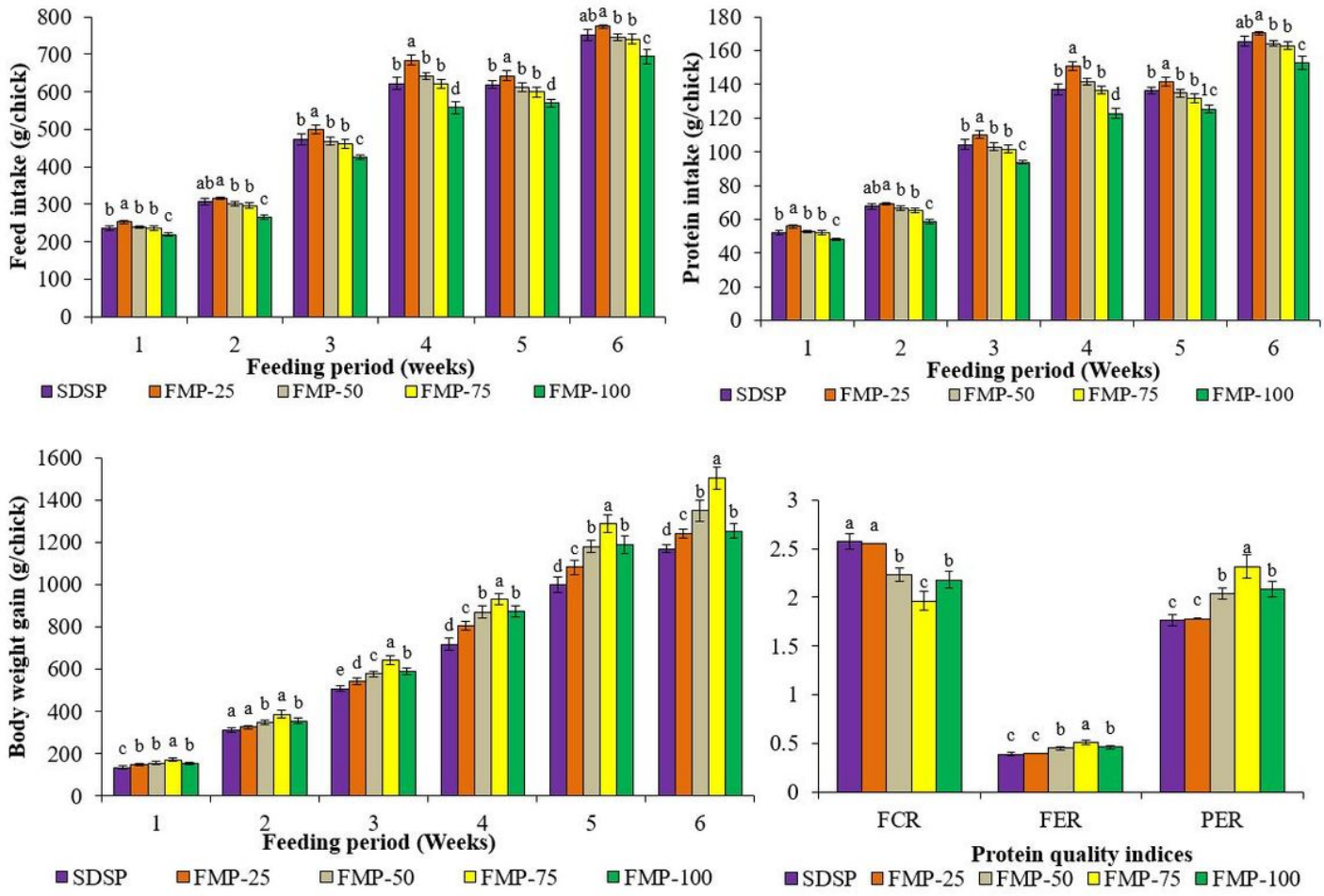

Figure 1

Daily feed intake (a), daily protein intake (b), weekly body weight gain (c), and FCR, FER, and PER (d) of the broiler chicks fed with SPSD and FWMPH at different levels (%). FWMPH: fermented meat processing wastewaters protein hydrolysate; SPSD: standard (soya protein concentrate); FMP-25: 25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH; FCR: Feed conversion ratio; FER: feed efficiency ratio; PER: protein efficiency ratio. Bars represent the standard deviation (n = 12).

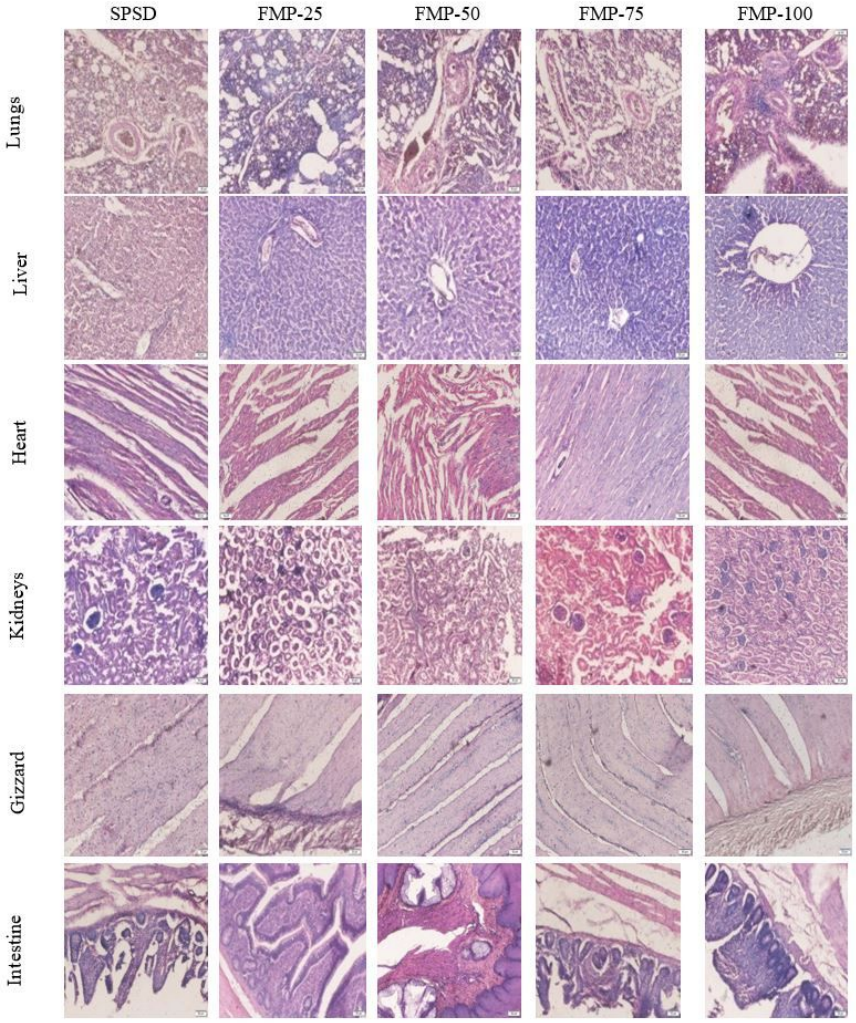

Figure 2

Histopathological images of lungs, liver, heart, intestine, gizzard, and kidneys of the broiler chicks fed with SPSD and FWMPH at different levels (%). Magnification 40x. FWMPH: fermented meat processing wastewaters protein hydrolysate; SPSD: standard (soya protein concentrate); FMP-25: 25% replacement with FWMPH; FMP-50: 50% replacement with FWMPH; FMP-75: 75% replacement with FWMPH; FMP-100: 100% replacement with FWMPH.