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Research Article
Growth Performance and Toxicological Assessments of Chicken Feather Protein Hydrolysate as Fish Meal Substitute in Rat Diet

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Abstract

Background and Objective: At present, Fish Meal (FM) and meat meal are the dominant animal protein sources for livestock feeds. However, these protein sources are not affordable for most livestock farmers, especially those in developing countries. Thus, it would be helpful to test other protein-based animal byproducts as alternative cheaper protein sources and as a solution to the overdependence of animal feeds on FM. The present study was undertaken to investigate the feasibility of replacing fish meal (FM) with chicken feather protein hydrolysate (CFPH) in rat diet. **Materials and Methods:** The changes in growth performance (after 4 weeks) and some tissue biochemical indices (after 7 weeks) were determined following feeding with iso-proteic and iso-energetic diets, in which 0 (control), 20, 40, 60, 80 and 100% of FM were replaced by CFPH (CFPH 0-100), respectively. **Results:** Food intake and weight gain showed progressive reduction with increasing proportion of CFPH in the diet increased. The growth parameters monitored were observed to decrease progressively with the increasing CFPH level in the diet. Plasma lipid concentrations were not significantly altered in the rats fed with 20 and 40% CFPH but were significantly lower in the rats fed with 60-100% CFPH compared to the rats fed with 100% FM. Fecal nitrogen excretion and plasma total protein concentration were significantly increased in the rats fed with 40-100% CFPH compared to rats fed 20 and 40% CFPH as well as 100% FM. Liver and kidney function indices monitored were not significantly altered in the rats fed CFPH up to 40% compared to the rats fed 100% FM. **Conclusion:** Based on the data generated from this study, it could be concluded that the inclusion of CFPH in rat diet up to 20% of the total dietary protein content is safe and has no drastic effect on the growth performance. Thus, feeding CFPH to animals could be a cost-effective solution to the challenge of waste feather disposal and could reduce the overdependence of livestock feed on FM, thereby ensuring sustainable livestock farming.

Key words: Animal diet, chicken feather waste, protein hydrolysate, dietary protein, growth performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

At present, Fish Meal (FM) and meat meal are the dominant animal protein sources for livestock feeds. However, these protein sources are not affordable for most livestock farmers, especially those in developing countries. Thus, it would be helpful to test other protein-based animal byproducts as alternative cheaper protein sources and as a solution to the overdependence of animal feeds on FM. The poultry industry has experienced tremendous growth over the past few years because of the increased demand for poultry and poultry-based products. Globally, approximately 20,800 million chickens are processed annually[1,2]. Typically, feathers constitute approximately 5-10% of the total weight of a live chicken; therefore, the weekly worldwide production of feather waste is approximately[1,2] 20 Mt. That is, the contribution of feather waste to the total solid waste is substantial.

Feathers are composed of a considerable amount (85-90%) of crude protein[3]. However, feather protein is not easily hydrolyzed by digestive enzymes in its natural state[3,4]. Consequently, in recent times, research efforts have increasingly focused on the possibility of processing feathers to enhance its digestibility. A breakthrough in this direction will not only provide an additional cheap source of animal protein for livestock feeding but also offer a sustainable strategy for mitigating the environmental consequences of increasing chicken feather waste from the ever-growing poultry industry.

The use of acids and alkalis for hydrolyzing feather biomass is a very typical method used in biomass transformation processes[5-8]. However, acid hydrolysis has been reported to cause the loss of some amino acids such as tryptophan[6]. On the other hand, the hydrolysis of feather biomass using alkalis is slower than that using acids; however, the use of alkalis often provides the advantage of a reduced loss of amino acids. Furthermore, studies have shown that feather protein hydrolysates obtained from alkali hydrolysis can be utilized as a growth substrate for the microbial production of important metabolites including carotenoids[6], polysaccharides[9], glutathione[9] and lactic acid[10].

So far, very few studies have evaluated the quality and safety of hydrolyzed feather protein hydrolysate in rat diet. However, hydrolyzed feather meal has been effectively utilized to replace FM in the diets of European seabass[11] and pigs[12] without any significant impairment on growth. The present authors are of the view that a positive assessment of chicken feather protein hydrolysate (CFPH) in terms of its nutritional performance and toxicity could serve as a cheap and accessible solution to the problem of malnutrition, especially among low-income groups. The present study was aimed at evaluating the effects of CFPH vis-à-vis its toxicity on the growth performance of growing albino rats by using biochemical parameters.

MATERIALS AND METHODS

Animal ethics: The study protocol was approved by the Animal Ethics Committee of the Department of Biological Science, Landmark University, Omu-Aran, Nigeria and was conducted in strict adherence to the NIH guidelines for the care and use of laboratory animals.

Chicken feather waste: White-colored chicken feather waste was collected from the slaughter house of the Landmark University Commercial Farm (Omu-Aran, Nigeria).

Preparation of chicken feather protein hydrolysate: Chicken feathers were washed with detergent, rinsed thoroughly with a copious amount of water and sun-dried. The dried feathers were ground into powder using a mechanical grinder. Three hundred gram (300 g) of the powdered feathers was weighed and extracted with a 1 mol L^{-1} NaOH solution (wt/vol, 3:10) for 6 h at room temperature. Thereafter, the resulting mixture was filtered using a clean dry muslin cloth to remove unhydrolyzed feathers. The CFPH was precipitated from the filtrate using 10% trichloroacetic acid. The precipitated CFPH was filtered using a clean dry muslin cloth and oven dried at 50°C. The dried CFPH was stored in a dried airtight container and stored in a cool dry place until it was required for further analysis.

Experimental diets: The six experimental diets used in this study were prepared based on the formulation of the American Institute of Nutrition[12]. The formulated diets were designated as A, B, C, D, E and F, with diet A serving as the control and containing 100% FM. In diets B, C, D, E and F, FM was replaced with 20, 40, 60, 80 and 100% CFPH, respectively and mineral, vitamin and fiber were added in equal amounts. After preparation, the diets were stored in dried airtight containers until use.

Animals and treatments: The study was conducted in the animal house of the Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria. Thirty-six weaned, male Swiss albino rats (age: 6 weeks, average body weight: 45-70 g) were used. Following an initial 2 week acclimatization period, the animals were divided into 6 groups (n = 6) and fed their respective diets (Table 1). They were kept in wooden cages with raised wire-gauze floors;

Table 1: Animal grouping and dietary regimen (g kg^{-1}, DM)

	Dietary group					
Feed composition (g kg^{-1}, DM)	A	B	C	D	E	F
Corn flour	600.0	600.0	600.0	600.0	600.0	600.0
Fish meal	200.0	160.0	120.0	80.0	40.0	0.0
Chicken feather protein hydrolysate	0.0	40.0	80.0	120.0	160.0	200.0
Mineral premix (AIN-76)[a]	30.0	30.0	30.0	30.0	30.0	30.0
Vitamin premix (AIN-76)[b]	10.0	10.0	10.0	10.0	10.0	10.0
Fiber	100.0	100.0	100.0	100.0	100.0	100.0
Groundnut cake	60.0	60.0	60.0	60.0	60.0	60.0

[a]Mineral premix (AIN-76) composed of Ca as $CaSO_4$ (5.2 g kg^{-1}), Ka as KCl (3.8 g kg^{-1}), Na as NaCl (1.1 g kg^{-1}), Mg as $MgSO_4$ (0.5 g kg^{-1}), Fe as $FeSO_4$ (34.25 mg kg^{-1}), Zn as $ZnSO_4$ (36.75 mg kg^{-1}), Mn as $MnSO_4$ (59.34 mg kg^{-1}), Cu as $CuSO_4$ (6.73 mg kg^{-1}), Co as $CoCl_2$ (0.03 mg kg^{-1}) and I as KI (0.21 mg kg^{-1}), [b]Vitamin premix (AIN-76) composed of vitamin A (4.0 IU g^{-1}), vitamin D_3 (1.0 IU g^{-1}), α-tocopherol (64.24 IU kg^{-1}), thiamine (5.90 mg kg^{-1}), riboflavin (6.29 mg kg^{-1}), niacin (30.15 mg kg^{-1}), pantothenic acid (15.26 mg kg^{-1}), choline (1040.0 mg kg^{-1}), pyridoxine (7.12 mg kg^{-1}), folic acid (2.10 mg kg^{-1}), biotin (0.21 mg kg^{-1}), vitamin B_{12} (10.10 mg kg^{-1}) and vitamin K (0.50 mg kg^{-1}). A, B, C, D, E and F represent the dietary fish meal/chicken feather protein hydrolysate ratio of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, respectively

the cages were maintained at a temperature of 25.7±2.3°C and a relative humidity of 45-60%, with 12 h light/dark cycles. The animals had unrestricted access to their feed and water *ad libitum* throughout the duration of the experiment. Daily records of body weight and food intake were monitored and fecal samples from each group were collected every week and stored at -4°C. After 7 weeks of the feeding trial, the rats were made to fast overnight and were anaesthetized with chloroform, after which they were decapitated by cervical dislocation. The blood from the rats was rapidly collected by direct heart puncture and the plasma was prepared and stored at -4°C until it was required for analysis. The brain, liver, heart, kidneys, spleen, lung, testes and intestine were collected, blotted in tissue paper, freed of fat and weighed. All the samples were stored at -4°C until analysis.

Proximate composition analysis: The unprocessed chicken feathers, CFPH and the respective experimental diets were analyzed for moisture, ash, fiber and crude fat using the methods recommended by the Association of Official Analytical Chemists (AOAC)[13]. Crude protein was determined by the Kjeldahl method[14]. Phosphorus content was determined based on the procedures described by Twine and Williams[15], whereas sodium and potassium were estimated by atomic absorption spectrometry, as detailed by Rowe[16]. Amino acid analysis of the samples was carried out according to the method of Ravindran *et al.*[17]. Fecal nitrogen content was determined using the procedure described by AOAC[13].

Growth performance: The average daily weight gain was determined as the average weight gain each day during the 4 week period. Food intake [diet (g)/(rat×day)] was determined from the average amount of diet consumed by each rat during the first 28 day period. Protein intake was calculated as the crude protein percentage of the food intake. The Protein Efficiency Ratio (PER) was calculated as:

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Average daily weight gain}}{\text{Protein intake}}$$

Food efficiency (%) was calculated as:

$$\text{Food efficiency (\%)} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}} \times 100$$

The Specific Growth Ratio (SGR) was calculated as:

$$\text{Specific Growth Ratio (SGR)} = \frac{\text{Average weight gain after 28 days}}{28}$$

Biochemical analysis: Lipid profile analyses of triglycerides, total cholesterol and High-Density Lipoprotein (HDL) cholesterol were carried out using commercial kits (Randox Laboratories Ltd., UK) according to the manufacturer's instructions. Low-Density Lipoprotein (LDL) cholesterol was estimated using the formula proposed by Friedewald *et al.*[18]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using the method of Reitman and Frankel[19]. The lactate dehydrogenase (LDH) activity was estimated based on the method described by Wroblewski and Ladue[20], whereas, the protein concentration was determined according to the method of Gornall *et al.*[21]. Plasma urea and creatinine concentrations were estimated using the methods of Marsh *et al.*[22] and Brod and Sirota[23], respectively.

Statistical analysis: The obtained results were expressed as mean±standard error of mean (SEM). The statistical analysis was carried out by one-way analysis of variance followed by Tukey's multiple comparison test using SPSS version 20. The $p<0.05$ was considered significant. All the graphs were plotted using GraphPad Prism.

RESULTS

Proximate composition: The sodium and potassium levels were considerably higher in the CFPH (0.15%±0.01 and 0.65%±0.01, respectively) than in the raw feathers (0.05%±0.0 and 0.38%±0.04, respectively) (Table 2). The moisture content of the CFPH-containing diets was significantly higher than that of the 100% FM diet. Crude protein level in both the CFPH-based diets and 100% FM diet were similar (Table 3). Diets containing CFPH levels of

Table 2: Proximate composition analysis of raw feathers and chicken feather protein hydrolysate

Parameters	Raw chicken feather	Chicken feather protein hydrolysate
Proximate composition (Weight (%))		
Moisture	11.50±0.5[a]	28.80±0.02[b]
Ash	1.33±0.03[a]	1.40±0.34[a]
Crude fat	0.93±0.01[a]	0.03±0.00[a]
Crude protein	75.80±0.1[a]	61.63±0.04[b]
Crude fiber	2.67±0.02[a]	3.10±0.7[a]
Elemental composition		
Phosphorous	0.47±0.01[a]	0.42±0.0[a]
Sodium	0.05±0.0[a]	0.15±0.01[b]
Potassium	0.38±0.04[a]	0.65±0.01[b]
Amino acids (g/100 g protein)		
Lysine	0.98[a]	0.93[a]
Threonine	4.72[a]	4.80[a]
Cysteine	4.33[a]	3.51[b]
Leucine	8.05[a]	7.80[a]
Isoleucine	4.93[a]	4.63[a]
Tryptophan	2.38[a]	0.65[b]
Methionine	0.68[a]	0.72[a]
Phenylalanine	4.73[a]	4.90[a]
Histidine	0.45[a]	0.48[a]
Valine	8.50[a]	8.63[a]
Arginine	5.90[a]	6.01[a]
Serine	13.00[a]	12.65[a]
Glycine	9.52[a]	9.55[a]

Values are given as Mean±SD of triplicate determinations. Values in the same row carrying different superscripts are significant ($p<0.05$)

Table 3: Chemical composition of experimental diets

	Dietary groups					
Parameters	A	B	C	D	E	F
Proximate composition (%)						
Moisture	7.30±1.0[a]	9.00±2.0	9.00±1.5	9.00±1.3	8.00±0.3	9.00±0.7
Ash	6.00±1.1[a]	7.00±0.1	6.00±0.5	6.00±0.6	7.00±0.0	7.00±0.3
Crude fat	2.80±0.02[b]	2.50±0.01[b]	2.30±0.01[a,b]	2.30±0.02[a,b]	2.20±0.02[a,b]	1.80±0.02[a]
Crude fiber	0.60±0.12[b]	0.50±0.11[b]	0.50±0.13[b]	0.60±1.0[b]	0.30±0.15[a]	0.30±0.09[a]
Crude protein	24.42±1.1[a]	23.83±0.2[a]	21.12±0.5[a]	20.96±1.2[a]	22.16±0.4[a]	23.86±0.0[a]
Nitrogen-free extract	58.88±5.2[a]	57.17±3.8[a]	61.08±5.5[a]	61.14±2.1[a]	60.34±5.8[a]	58.04±4.9[a]
Energy (kJ/100 g)	1499.55[a]	1449.76[a]	1462.31[a]	1460.63[a]	1463.56[a]	1438.46[a]
Protein/calorie ratio (g protein/100 kcal)						
Amino acids (g/100 g protein)	6.81[a]	6.88[a]	6.04[a]	6.00[a]	6.34[a]	6.94[a]
Lysine	2.56[a]	0.58[b]	0.42[b,c]	0.33[c]	0.33[c]	0.31[c]
Threonine	4.50	4.52	4.38	4.35	4.35	4.33
Cysteine	1.02[a]	2.85[a]	3.27[b]	3.33[b]	3.50[b]	3.30[b]
Leucine	6.88	6.55	7.61	7.53	7.83	7.82
Isoleucine	4.58	4.55	4.38	4.53	4.52	4.50
Tryptophan	2.56[a]	1.01[b]	0.62[c]	0.55[c]	0.48[c]	0.48[c]
Methionine	2.79[a]	1.53[b]	0.88[c]	0.85[c]	0.81[c]	0.77[c]
Phenylalanine	5.53	5.50	5.33	5.35	5.30	5.22
Histidine	3.03[a]	2.82[a]	0.73[b]	0.65[b]	0.62[b]	0.63[b]
Valine	9.55	9.33	9.05	9.03	8.93	8.88
Arginine	4.83	4.85	4.55	4.33	4.55	4.52
Serine	12.58	12.80	12.85	13.20	13.20	13.05
Glycine	10.20	10.21	9.55	9.80	9.85	9.85

Values are given as Mean±SD of triplicate determinations. Values in the same row carrying different superscripts are significant ($p<0.05$), A, B, C, D, E and F represent the dietary fish meal/chicken feather protein hydrolysate ratio of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, respectively

60% or above had significantly higher content of cysteine than the 100% FM diet and 20% CFPH diet. However, diets containing CFPH had significantly lower contents of lysine, methionine, tryptophan and histidine than the 100% FM diet (Table 3).

Growth response and growth parameters: The body weight of the animals was observed to decrease as the percentage of CFPH in the diets increased (Fig. 1). The growth parameters obtained after the first 28 days of feeding showed that the mean body weight gain was highest in the rats fed with 100% FM, followed by those fed with 20% CFPH. Rats fed with 100% CFPH had the lowest weekly mean body weight gain (Table 4). Food intake in the rats fed with 20 and 40% CFPH was similar to that in the rats fed with 100% FM; however, food intake in the rats fed with 60, 80 and 100% CFPH was significantly lower than that in the rats fed with 100% FM. The PER in the rats fed with 20% CFPH was similar to that in the rats fed with 100% FM; however, the PER in the rats fed with 40, 60, 80 and 100% CFPH was significantly lower than that in the rats fed with 100% FM. In contrast, the food efficiency, SGR and FCR were significantly lower in the CFPH-fed rats than in the rats fed with 100% FM (Table 4).

Relative organ weights: The relative heart and spleen weights were not significantly affected across the dietary groups. The increase in the relative weights of the liver, kidney, lung, brain, testes and intestine in the rats fed with 40, 60 and 100% CFPH was significant compared to the rats fed with 100% FM (Table 5).

Lipid profile: Plasma triglyceride (Fig. 2a), total cholesterol (Fig. 2b) and LDL cholesterol (Fig. 2c) concentrations in the rats fed with 20 and 40% CFPH were not significantly different from the corresponding concentrations in the rats fed with 100% FM; however, these concentrations in the rats fed with 60-100% CFPH were significantly

Table 4: Growth parameters of rats fed with experimental diets for 4 weeks

Dietary groups	A	B	C	D	E	F
Final body weight (g)	189.50±28.6^{a}	143.36±17.06^{b}	89.13±13.15^{C}	93.99±19.6^{c}	68.79±6.35^{d}	72.00±2.3^{d}
Initial body weight (g)	57.23±1.56^{a,b}	56.20±1.64^{a,b}	46.97±3.28^{a}	57.98±3.44^{a,b}	52.83±4.6^{a}	64.15±5.63^{b}
Average daily weight gain (g)	4.72±0.97^{a}	3.11±0.57^{b}	1.47±0.38^{c}	1.28±0.59^{c}	0.50±0.1^{d}	-0.13±0.0^{e}
Food intake (g)	14.29±1.3^{a}	13.81±1.1^{a}	10.00±1.2^{a,b}	9.52±1.01^{b}	7.14±0.8^{b}	3.57±0.5^{c}
Protein intake (g)	3.43±0.4^{a}	3.01±1.0^{a}	2.10±0.3^{b}	2.00±0.3^{b}	1.57±0.5^{b}	0.86±0.1^{c}
Protein efficiency ratio	1.38±0.1^{a}	1.03±0.1^{a}	0.70±0.1^{b}	0.64±0.1^{b}	0.32±0.1^{c}	-0.15±0.0^{d}
Food efficiency (%)	36.60±2.2^{a}	22.52±3.5^{b}	14.70±1.2^{c}	13.91±1.5^{c}	7.00±1.3^{d}	-3.63±1.2^{e}
Specific growth ratio	4.54±1.1^{a}	2.87±0.7^{b}	2.29±0.6^{b}	1.73±0.9^{c}	0.94±0.3^{d}	0.40±0.1^{e}
Feed conversion ratio	2.73±1.1^{a}	5.59±1.3^{b}	6.80±1.3 ^{b}	7.34±1.8^{b}	14. 28±2.3 ^{c}	-
AEC/FER	0.37±0.1^{a}	0.18±0.01^{b}	0.15±0.01^{c}	0.14±0.1^{c}	0.07±0.1^{d}	-
Survival (%)	100.0	100.0	83.3	83.3	66.7	33.3

Values are given as Mean±SD of triplicate determinations. Values in the same row carrying different superscripts are significant ($p<0.05$), A, B, C, D, E and F represent the dietary fish meal/chicken feather protein hydrolysate ratio of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, respectively

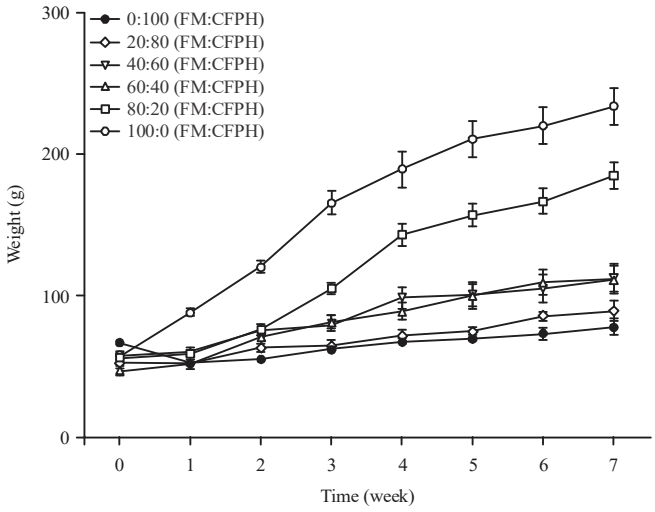

Fig. 1: Growth response of rats fed with different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations, FM: Fish meal, CFPH: Chicken feather protein hydrolysate

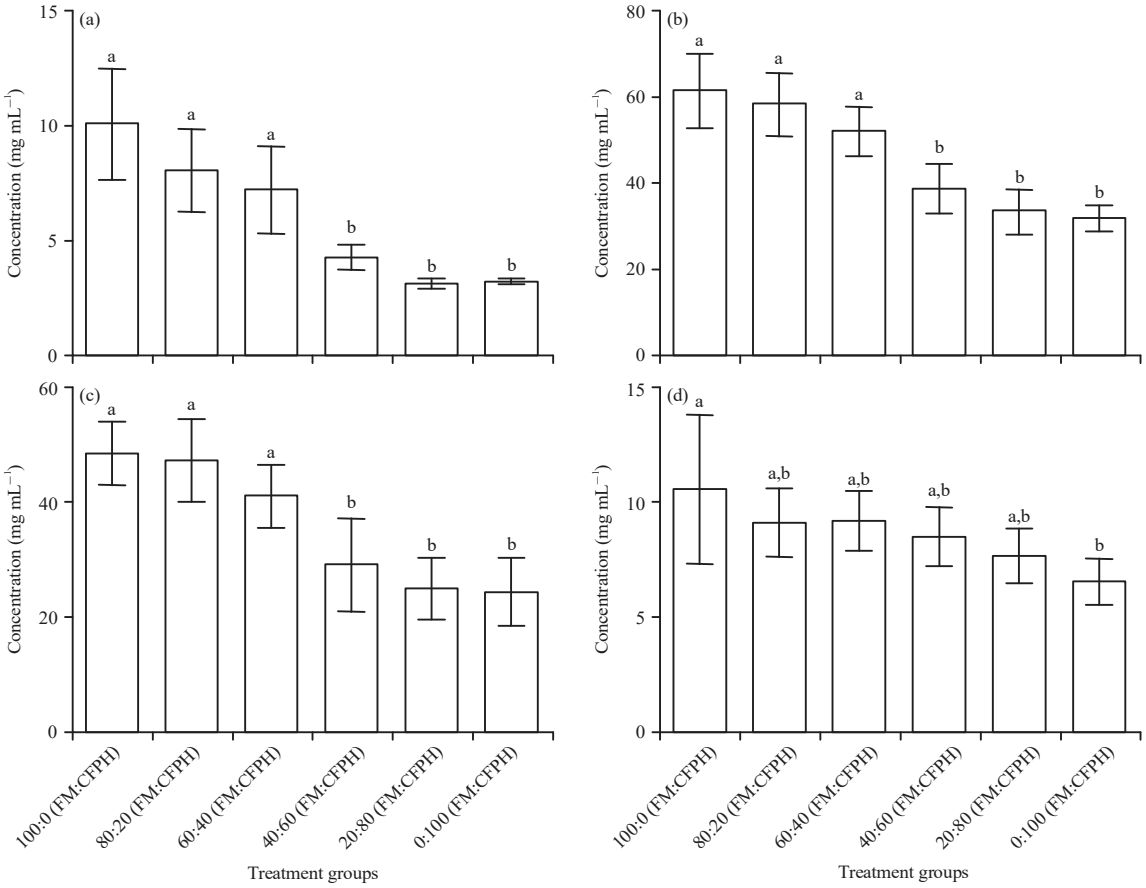

Fig. 2(a-d): (a) Plasma triglyceride, (b) Total cholesterol, (c) Low-density lipoprotein cholesterol and (d) High-density lipoprotein cholesterol concentrations of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations, different superscripts represent significant differences ($p<0.05$), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

Table 5: Relative organ weights of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Parameters	A	B	C	D	E	F
Liver	0.040±0.001^{a}	0.050±0.001^{a}	0.050±0.001^{a}	0.050±0.001^{a}	0.060±0.001^{a,b}	0.070±0.001^{b}
Heart	0.003±0.0^{a}	0.003±0.0^{a}	0.003±0.0^{a}	0.003±0.00^{a}	0.003±0.0^{a}	0.004±0.0^{a}
Kidneys	0.005±0 0^{a}	0.006±0.0^{a}	0.007±0.0^{a}	0.008±0.0^{b}	0.010±0.0^{b}	0.010±0.0^{b}
Lung	0.006±0.0^{a}	0.007±0.0^{a}	0.010±0.0^{b}	0.012±0.0^{b}	0.011±0.0^{b}	0.012±0.0^{b}
Brain	0.006±0.0^{a}	0.010±0.0^{b}	0.011±0.0^{b}	0.013±0.0^{b,c}	0.015±0.0^{c}	0.015±0.0^{c}
Intestine	0.080±0.00^{a}	0.100±0.0^{a}	0.130±0.0^{b}	0.150±0.0^{b,c}	0.170±0.0^{c}	0.170±0.0^{c}
Spleen	0.005±0.0^{a}	0.005±0.0^{a}	0.005±0.0^{a}	0.005±0.0^{a}	0.005±0.0^{a}	0.006±0.0^{a}
Testes	0.010±0.0^{a}	0.011±0.0^{a}	0.011±0.0^{a}	0.012±0.0^{a}	0.017±0.0^{b}	0.018±0.0^{b}

Values are given as Mean±SEM of 2-6 determinations, values in the same row carrying different superscripts are significant ($p<0.05$), A, B, C, D, E and F represent the dietary fish meal/chicken feather protein hydrolysate ratio of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, respectively

lower than the corresponding concentrations in the rats fed with 100% FM (Fig. 2d).

Fecal nitrogen and glucose concentration: Fecal nitrogen excretion was significantly higher in the rats fed with 40, 60, 80 and 100% CFPH than in the rats fed with 100% FM. The nitrogen level in the feces of the rats fed with 20% CFPH was not significantly different from that of the rats fed with 100% FM (Fig. 3a).

Tissue protein: The plasma protein concentration in the rats fed with 20 and 40% CFPH was not significantly different from that in the rats fed with 100% FM. However, the plasma protein level in the rats fed with 60, 80 and 100% CFPH was significantly greater than that in the rats fed with 100% FM (Fig. 4a). The total protein concentrations for the liver (Fig. 4b) and heart (Fig. 4c) in the rats fed with 20% CFPH were similar to those in the rats fed with 100% FM; however, these concentrations in the

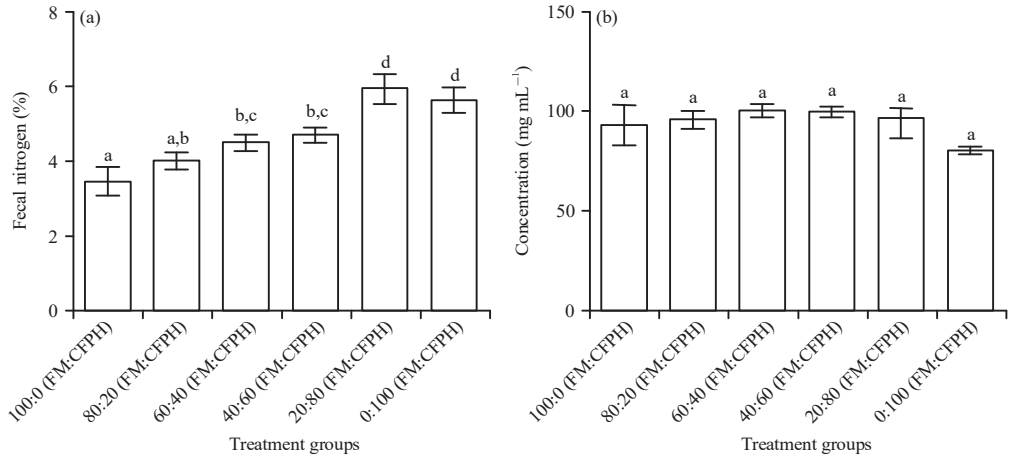

Fig. 3(a-b): (a) Fecal nitrogen and (b) Glucose concentration of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations. Different superscripts represent significant differences (p<0.05), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

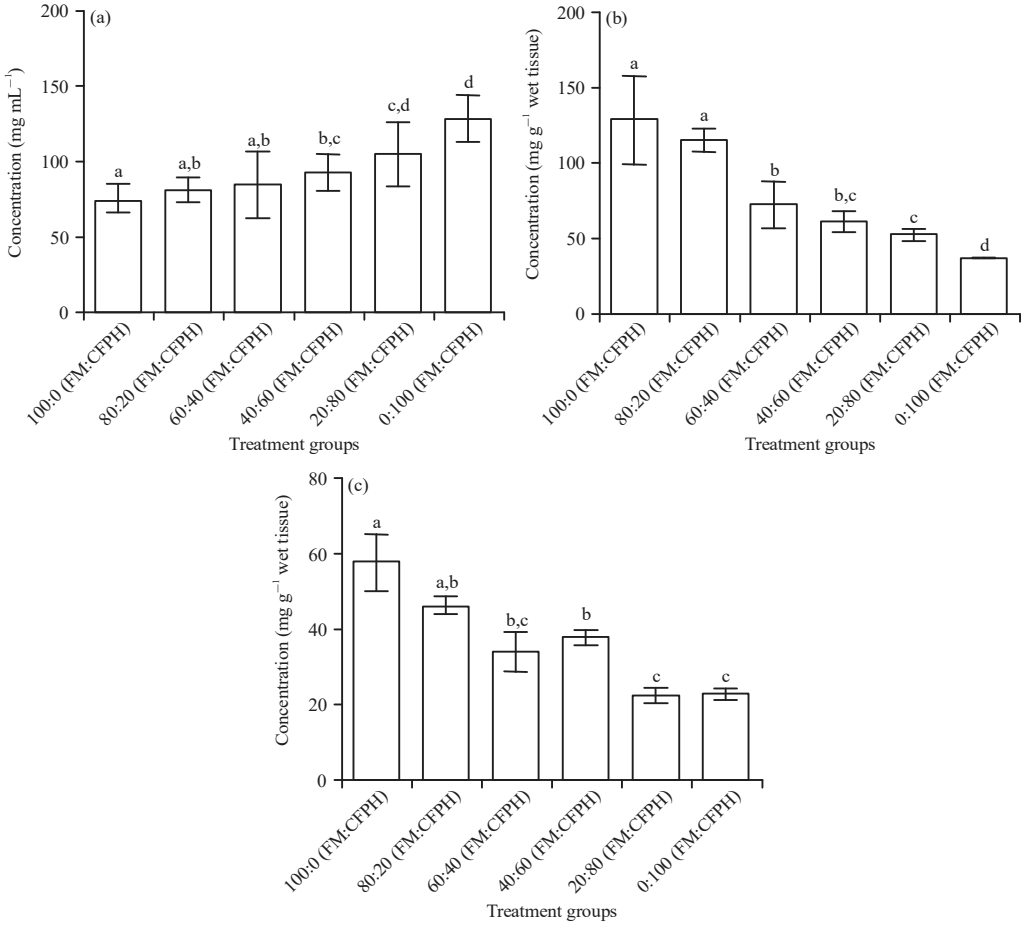

Fig. 4(a-c): (a) Plasma, (b) Liver and (c) Heart protein concentrations of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations, different superscripts represent significant differences (p<0.05), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

rats fed with 40, 60, 80 and 100% CFPH were significantly lower than those in the rats fed with 100% FM.

Tissue enzyme activity: Plasma ALT (Fig. 5a) and AST (Fig. 5b) activities as well as liver ALT (Fig. 5c) and AST (Fig. 5d) activities in the CFPH-fed rats were not significantly different from those in the rats fed with 100% FM. In addition, plasma (Fig. 6a), liver (Fig. 6b) and heart (Fig. 6c) LDH activities in the CFPH-fed dietary groups were not significantly different from those in the rats fed with 100% FM.

Kidney function parameters: Plasma creatinine (Fig. 7a) and urea (Fig. 7b) concentrations in the rats fed with 100% CFPH were significantly higher than those in the rats fed with 100% FM. However, the creatinine and urea levels in the rats fed with 20, 40, 60 and 80% CFPH were not significantly different from those in the rats fed with 100% FM.

DISCUSSION

The quality of a protein is determined based on the ability of the protein to provide an adequate amount of essential amino acids to the organism. Feather meal diets have been reported to be of low quality in terms of their protein component[24]. The low content of essential amino acids such as methionine, lysine, histidine and tryptophan in CFPH diets may have contributed to the decrease in the average daily weight gain and low growth response observed in the rats fed with the CFPH diets. Although, the digestibility and bioavailability of the test diets were not determined in this study, the high fecal nitrogen levels observed in the CFPH-fed rats in this study could bean indication of the low digestibility and bioavailability of CFPH. These observations further stress the need for further hydrolysis of the keratin molecule. Further hydrolysis of

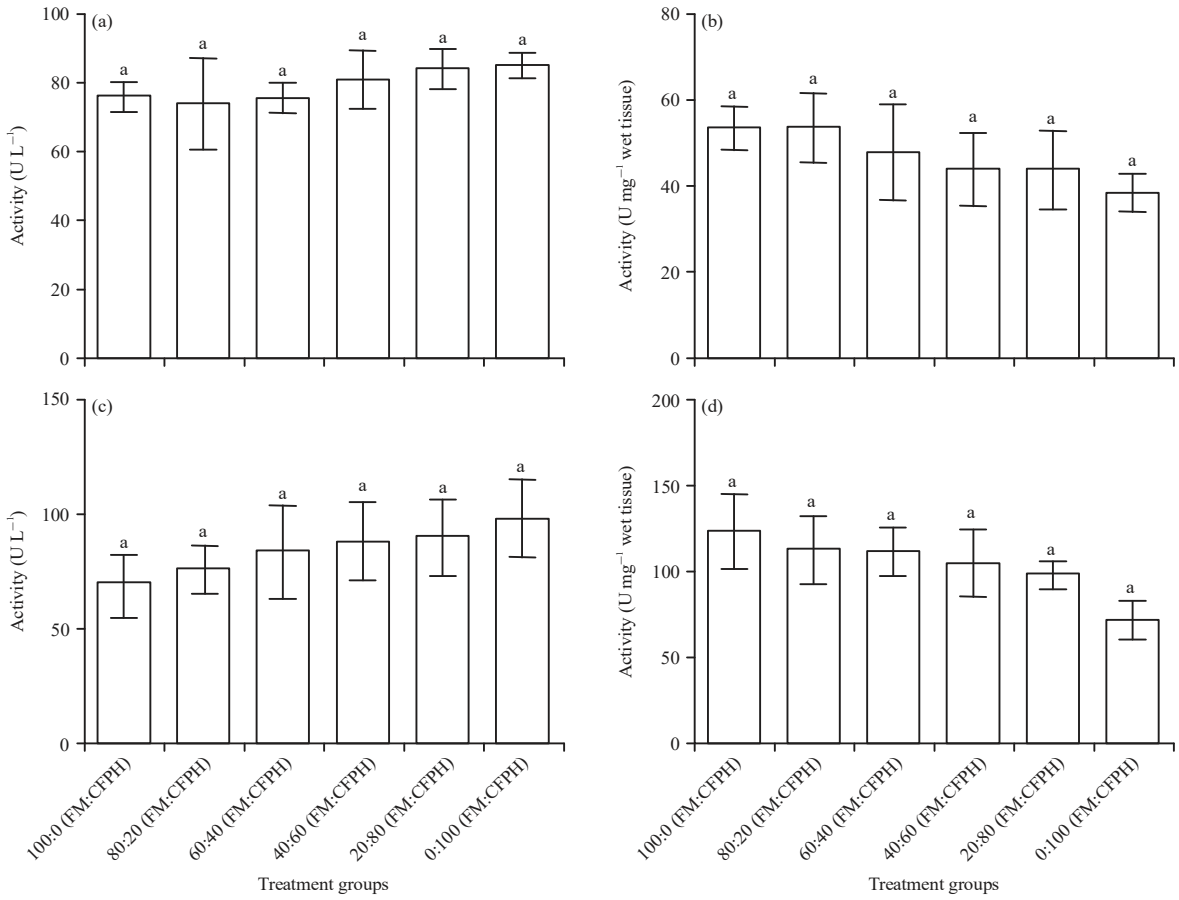

Fig. 5(a-d): (a) Plasma alanine aminotransferase (ALT) and (b) Aspartate aminotransferase (AST) and (c) Liver ALT and (d) AST activities of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations, different superscripts represent significant differences ($p<0.05$), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

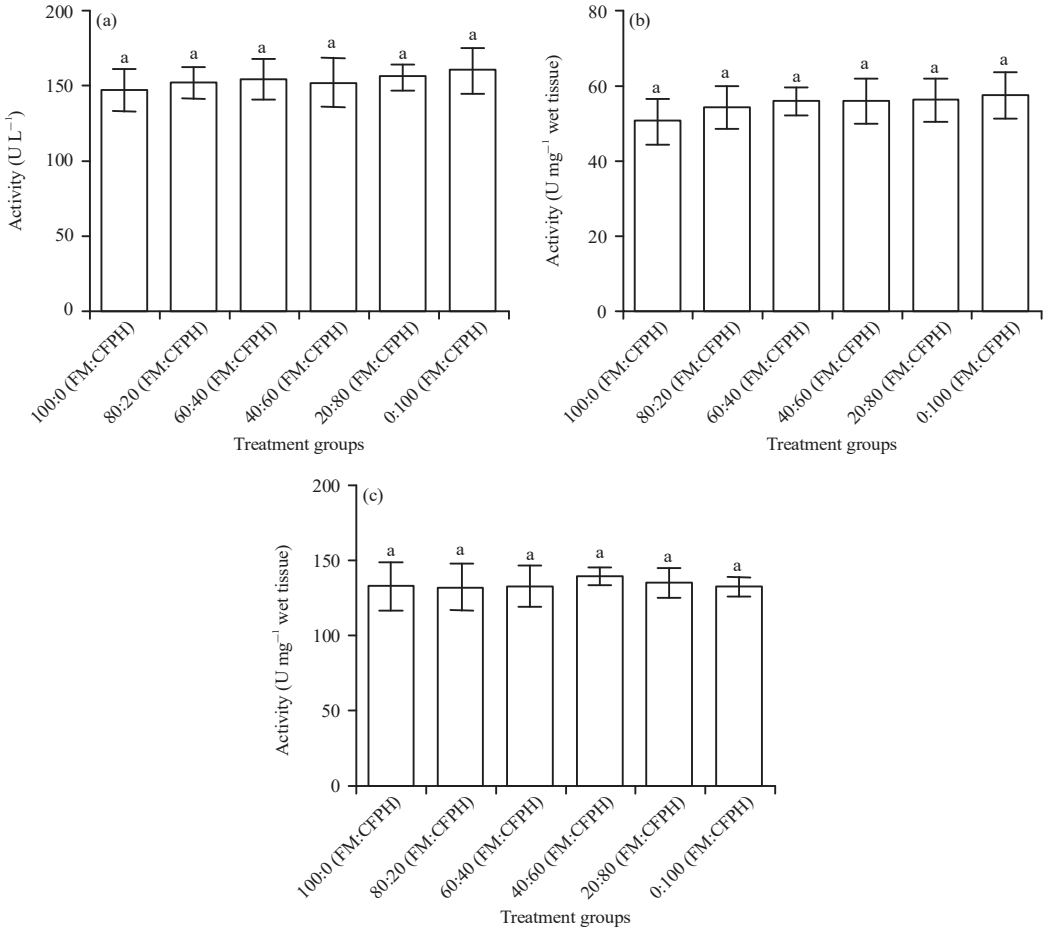

Fig. 6(a-c): (a) Plasma, (b) Liver and (c) Heart lactate dehydrogenase (LDH) activities of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 different determinations, different superscripts represent significant differences ($p<0.05$), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

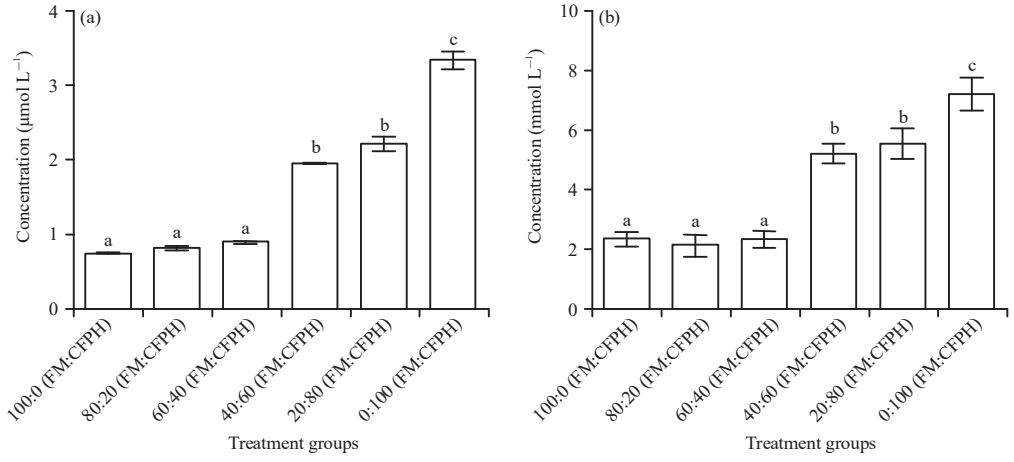

Fig. 7(a-b): (a) Plasma creatinine and (b) Urea concentrations of rats fed with diets containing different proportions of chicken feather protein hydrolysate as a replacement for fish meal for 7 weeks

Values are given as Mean±SEM of 2-6 determinations, different superscripts represent significant differences ($p<0.05$), FM: Fish meal, CFPH: Chicken feather protein hydrolysate

CFPH into peptides and amino acids using microorganisms or enzymes may be necessary to enhance its digestibility and bioavailability *in vivo*[11].

The reduction in tryptophan content in CFPH compared to that in raw chicken feather observed in this study is not unexpected given that the hydrolysate was obtained through treatment with sodium hydroxide. The alkali hydrolysis of feathers has been reported to cause the loss of amino acids such as tryptophan[25-27]. Thus, the inferior growth response and weight gain observed in all the diets containing CFPH can be attributed in part to the deficiency of some amino acids that are considered to be indispensable[28]. Reports from separate studies by Kim *et al*.[29], Coward-Kelly *et al*.[30] and Murphy *et al*.[31] on the nutritional value of powdered chicken feathers for growing rats have shown that the feather protein supports moderate growth when it is supplemented with methionine, lysine, histidine and tryptophan.

In this study, the inability of the rats fed with diets containing a high proportion of CFPH to consume the amount of food necessary to sustain their body requirement for growth and development may have been the reason for the mortality recorded in these groups. Thus, it can be recommended that the inclusion of CFPH in rat feeds should not exceed 20% of the total dietary protein content. A similar decrease in feed intake with a concomitant decrease in the weight gain was observed by El Boushy *et al*.[28] when feather meal was fed to Nile tilapia fish in comparison with casein. On the other hand, the loss in the body weight and average daily weight gain of rats fed with the CFPH diets can be an indication of muscle degradation because of loss or degradation of structural proteins. Muscle wasting is usually caused by the gluconeogenic synthesis of glucose from lipids and proteinaceous materials as a compensatory strategy for the non availability of energy from dietary sources[32].

Data obtained from this study showed a significant decrease in plasma triglyceride and total cholesterol and a concomitant increase in fecal nitrogen excretion in the rats fed with diets containing CFPH levels of above 40% compared to the corresponding levels in the rats fed with 100% FM. This is the first report on the effect of CFPH on plasma lipids in rats. However, it has been established that dietary proteins can produce significant improvement in blood lipid profiles. Several dietary proteins such as soy protein, whey and fish protein have been reported to exhibit hypocholesterolemic activity[33-35]. Mammals have been reported to adapt to food deprivation by increasing the rate of fat mobilization from the adipocytes to limit the utilization of body proteins as an energy source[36]. However, this adaptive strategy does not function in chronic or advanced stage of starvation, during which a progressive increase in protein utilization occurs[37-39]. Thus, the significant decrease in body mass loss and increase in fecal nitrogen excretion positively correlate with the decrease in the plasma triglyceride concentration[37,38].

To ascertain the potential toxicity of CFPH used in this study, AST and ALT activities in the plasma and liver were evaluated. In addition, the LDH activities of the plasma, liver and heart are determined. However, our findings show no significant effect of the dietary treatments on the plasma, hepatic and heart aminotransferases and LDH, suggesting that there has been no hepatic or cardiac failure. This assumption is further reinforced by the data on the relative organ weights, which show no significant difference in the heart, kidney, lung, spleen, intestine and brain across the groups. The relative organ weight is often used as an important endpoint for identifying potentially harmful effects of food or chemical substances in rats[40,41]. Plasma urea and creatinine concentrations in rats fed with diets containing CFPH levels of over 40% are significantly higher than the corresponding concentrations in the plasma of the rats fed with 100% FM. This is indicative of the increased muscle protein breakdown in these animals. This breakdown is caused by the switch to the use of proteins for energy production in chronic starvation cases because of the reduced feed intake in the rats fed with high levels of CFPH. Thus, the increased plasma creatinine level in these animals may be a consequence of a very high synthesis rate in relation to the clearance of creatinine by the kidney (i.e., a low glomerular filtration rate). In addition, the increased plasma urea levels recorded in the rats fed with diets containing CFPH levels of above 40% provide further justification to suggest a reduced or compromised renal function in these animals.

CONCLUSION

The results obtained showed that the inclusion of a high proportion of chicken feather protein hydrolysate as a replacement for fish meal shows potential symptoms of toxicity in rats, as manifested in the increased relative weights of the liver, kidney, lung, brain, intestine and testis; high fecal nitrogen excretion and high creatinine and urea concentrations in plasma. However, the addition of chicken feather protein hydrolysate up to 20% of the total dietary protein content as a replacement for fish meal is considered safe and has no drastic effect on growth performance.

However, for best results, supplementation with methionine, lysine, histidine and tryptophan is necessary. Thus, feeding chicken feathers to animals could be a cost-effective solution to the challenge of waste feather disposal and could reduce the overdependence of livestock feed on fish meal, thereby ensuring sustainable livestock farming.

SIGNIFICANCE STATEMENT

This study investigates the potential of chicken feather protein hydrolysate (CFPH), in terms of its nutritional performance and toxicity, as a cheap and accessible solution to the problem of malnutrition. In addition, the study provides a viable and useful way of dealing with the environmental concern associated with increasing chicken feather wastes.

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