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Effect of processed fish wastes supplementation on blood biochemical and meat composition of broiler chicken

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Summary

The experiment was conducted on forty five broiler chickens at 4 weeks of age, for a period of three weeks, to assess the effect of complete replacement of fish meal with processed fish wastes (fish waste acid silage and surimi waste powder) on the blood protein, lipid and antioxidant status in broiler chicken, even though an earlier study indicated that this replacement induced normal growth rate and feed efficiency in broilers. The quality of the meat and involvement of liver enzymes were also assessed in the study. At three weeks of age, they were randomly divided into three groups viz., GI, GII and GIII of 15 birds each. Two experimental diets (D2 and D3) were prepared by replacing 100 percent of dried unsalted fish (animal protein) in the finisher ration of the control diet (D1) by processed fish waste acid silage (D2) and surimi waste powder (D3). All diets were made isocaloric and isonitrogenous. GI, GII and GIII were fed with D1, D2 and D3 diets, respectively from the 4th to the 7th week of age. Blood samples were analysed to evaluate the status of proteins, lipids, liver enzymes, antioxidants and minerals. Meat sample from the pectoral region was utilized for assessing the meat quality. The serum total protein, albumin and globulin were similar in all the groups and they expressed a positive correlation with age. Except for a decrease in HDL-cholesterol in GII birds, the total lipids, triglycerides, total cholesterol and VLDL-cholesterol were similar in all the groups at the end of the study. The liver enzymes (ALT and AST) expressed a similar level in all the groups but increased with increase of age. The antioxidants (SOD, Catalase, GSH and LPO) indicated an increased level with increase of age, but were similar in all the groups. The major elements (Na, K, Ca and Mg) and iron were similar in all the groups and were within the normal levels. The results indicated that processed fish wastes could be used for complete replacement of animal protein requirements in broiler feed, as it adequately meets the nutritional requirements, alleviates stress, has no toxicity and also maintains the meat quality.

Key words: Broiler chicken, Fish wastes, Blood, Meat

Introduction

The poultry industry has undergone a revolutionary change within the last three decades and has become the most dynamic sector of the livestock industry. Poultry meat and eggs are an important part of the diet of the average family. Fish farming produces large amounts of by-products during its commercial processing, which are potential sources of pollution if dumped at sea or discarded offshore. It is estimated that one million tonnes of fish wastes are produced annually in India, and of this waste, surimi waste comprises about 2000 tonnes (Raghavan, 2007). The by-products from the fishing industry and fish farming have been shown to be a valuable animal protein source. Hence, studies were conducted to replace the animal protein source in poultry food with locally processed fish wastes, so as to reduce feed cost and mitigate pollution without compromising the growth and wellness of the birds (Darsana *et al*., 2009).

Fish waste acid silage (fish waste mixed in 3 per cent (w/v) formic acid and dried)

and surimi waste powder (fish waste cooked in 20 per cent (w/v) water and dried) are two products developed from fish waste obtained during the processing of *Nemiterus japonicas*, Japanese thread fin bream, by the Central Institute of Fisheries Technology (CIFT), Kochi. Our previous study (Darsana *et al*., 2009) reported that complete replacement of the animal proteins (fish meal) in the finisher ration of broiler chicken with processed fish wastes reduced the feed cost without compromising the nutritional status, feed efficiency and overall performance, but no information is available concerning its influence on protein, fat and mineral metabolism. Besides, its influence on antioxidant enzymes, liver enzymes, proximate composition and the cholesterol content of meat are also lacking. The present study was undertaken to address the influence of 100 per cent replacement of fish meal in the finisher ration of broiler chicken with fish waste silage/surimi waste powder on the above parameters.

Materials and Methods

Forty five, day old broiler chickens (Vencob strain) purchased from Coastal Krishna Hatcheries Thrissur, Kerala, India, were reared under standard managemental condition in a battery brooder. They were given a commercial broiler starter ration of Bureau of Indian Standards (BIS, 1992) specification for the first three weeks. At three weeks of age, they were randomly divided into three groups ie., GI, GII and GIII comprising 15 birds each. Two experimental diets (D2 and D3) were prepared by replacing 100 percent of the dried unsalted fish in the finisher ration of the control diet (D1) by processed fish waste acid silage (D2) and surimi waste powder (D3), and were fed to birds in GII and GIII, respectively. The birds in GI were fed with a standard broiler ration of BIS specification (D1). The proximate analysis of the processed fish waste acid silage and surimi waste powder was carried out and all of the diets were formulated to be isocaloric and isonitrogenous. Blood samples were collected from the wing vein, with or without anticoagulants, at weekly intervals from the 4th to the 7th week of age from all of the groups. After the final blood collection, the birds were sacrificed by cervical dislocation and a piece of meat (10 g) was excised from the cranial aspect of the pectoral region for proximate analysis and cholesterol estimation.

Serum total proteins were estimated by Biuret method (Henry *et al*., 1957), serum albumin by the method of Doumas *et al*. (1971) and the serum globulin was calculated as the difference between the serum proteins and the albumin contents.

The serum total lipids (Phosphovanillne method, Zoeliner, 1962), serum HDL-cholesterol (Phosphotungstate magnesium chloride method, Bachorik *et al*., 1976); serum total cholesterol (CHOD-PAP method, Richmond, 1973) and serum triglycerides (Schettler and Nussel, 1975) were determined. The serum VLDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al*., 1972). The liver function was determined by evaluating serum ALT and AST by UV-kinetic method (Bergmeyer, 1974) using Automatic Biochemistry Analyser (Shinnowa BS-3000P). The antioxidant status was evaluated by determining the activities of the blood catalase (Aebi, 1974), serum reduced glutathione (GSH, Moron *et al*., 1979), blood superoxide dismutase (SOD, Winterbourn *et al*., 1975) and serum lipid preoxidation (LPO, Ohkawa *et al*., 1979) using Automatic Biochemistry Analyser (Shinnowa BS-3000P). The serum mineral profiles (sodium, potassium, calcium, magnesium and iron) were estimated using the Atomic Absorption Spectrophotometer (Perkin Elmer-2330). The quality of the meat was evaluated through proximate analysis (AOAC, 1990) and meat cholesterol content (HOD-PAP method, Richmond, 1973).

The data were analysed by analysis of variance and paired t-test (Snedecor and Cochran, 1994) for test of significance using the statistical package (SPSS 10.0)

Results

The proximate analysis indicated the content of moisture (18% and 5.29%), dry

matter (82% and 94.17%), total ash (35.40% and 40.19%), acid soluble ash (0.514% and 0.341%), crude protein (50.3% and 49.54%) and crude fat (6.28% and 6.55%), respectively in processed fish waste acid silage and surimi waste. The ingredients of the diets (D1, D2 and D3) are presented in Table 1. The serum total proteins, albumin and globulin concentrations are presented in Table 2. The total proteins and albumin levels expressed no significant (P>0.05) difference between any of the groups, but the levels increased significantly (P<0.05) with the increase of age in all of the groups. Though at the fourth week of age the globulin content in GII and GIII was similar and significantly (P>0.05) lower in GI, the levels later increased and there was no significant (P>0.05) difference between any of the groups. A significant (P<0.05) increase with an increase in age was also demonstrated in the case of globulin.

Table 1: Ingredient composition of experimental diets

Ingredient (%)	D1	D2	D3
Maize	61.1	63.5	62.2
De oiled rice bran	9.3	4.8	4.1
Soya bean meal	18.4	20.5	22.3
Unsalted dried fish	10	-	-
Acid silage (fish waste)	-	10	-
Surimi waste power	-	-	10
Dicalcium phosphate	0.8	0.8	0.8
Methionine	0.3	0.3	0.3
Salt	0.006	0.016	0.025
Total	100.00	100.00	100.00
Added g/100 kg feed			
Vitamin mixture	10	10	10
L Lysine	100	100	100
Choline chloride	102	120	120
Coccidiostat	50	50	50
Toxin binder	250	250	250
B-Complex powder	50	50	50

The levels of serum total lipids, triglycerides, total cholesterol, HDL-cholesterol and VLDL-cholesterol are given in Table 3. The total lipids and triglycerides revealed no significant (P>0.05) difference between any of the groups. Both of these constituents increased significantly (P<0.05) with the increase of age, but the elevated triglyceride level in GIII was only numerically higher at the fifth week of age compared to the previous week. Throughout the study the total cholesterol content did not express any significant (P>0.05) difference between any of the groups, but did increase significantly (P<0.05) with the increase of age. Except for a significant (P<0.05) decrease in the HDL-cholesterol level in GII compared to GI at the end of the study, the levels were not significantly (P>0.05) different between any of the groups during the rest of the study period. The VLDL-cholesterol level was not significantly (P>0.05) different between any of the groups, but expressed an increase with the increase of age.

The ALT (Fig. 1) and AST (Fig. 2) concentrations were similar in all the groups throughout the study period. These enzymes expressed a significant (P<0.05) increase with the advancement of age. The antioxidant status in terms of activities of SOD, catalase, GSH and LPO (Table 4) indicated a significant (P<0.05) increase with the increase of age and they were similar in all of the groups.

The blood mineral status of the birds is given in Table 5. The major elements (Na, K, Ca and Mg) and iron expressed no significant (P>0.05) difference between any of the groups, except for an increase and then a fall in magnesium during the fifth and sixth week of age, respectively in the GII and GIII birds. But at the end of the study, all of the groups had a similar mineral concentration and were significantly (P<0.05) higher than the concentration at the beginning of the experiment.

The mean values of the meat crude protein, crude fat and total ash expressed no significant (P<0.05) difference between the control and the treatment groups (Fig. 3). The meat cholesterol content of GI, GII and GIII (72.07 ± 2.53, 73.00 ± 2.51 and 72.00 ± 1.75 mg/dl, respectively) expressed no significant (P<0.05) between them.

Discussion

Unsalted dried fish is added as a source of animal protein in the poultry diet (D1). The crude protein content of the processed fish waste acid silage and surimi waste was higher than that of unsalted dried fish (45%), but the crude fat content was marginally lower than that of unsalted dried fish (7%). These values indicated that the processed

Table 2: Effect of dietary supplementation of processed fish waste on blood protein (g/dl) profile of broiler chicken (mean ± SE, n = 15)

Group	Age (wks)				Age (wks)				Age (wks)			
	4	5	6	7	4	5	6	7	4	5	6	7
	Total protein				Albumin				Globulin			
GI	1.95^{a} ±0.12	2.20^{aA} ±0.16	2.54^{aA} ±0.12	3.05^{aA} ±0.12	1.24^{a} ±0.04	1.18^{aA} ±0.07	1.3^{aA} ±0.06	1.59^{aA} ±0.05	1.07^{a} ±0.08	1.02^{a} ±0.01	1.23^{aA} ±0.06	1.45^{aA} ±0.06
GII	1.69^{a} ±0.11	2.05^{aA} ±0.11	2.34^{aA} ±0.13	2.86^{aA} ±0.11	1.16^{a} ±0.04	1.07^{aA} ±0.06	1.17^{aA} ±0.07	1.49^{aA} ± 0.07	0.80^{b} ±0.06	0.99^{aA} ±0.06	1.17^{aA} ±0.06	1.37^{aA} ±0.05
GIII	1.67^{a} ±0.09	2.01^{aA} ±0.09	2.46^{aA} ±0.11	3.01^{aA} ±0.11	1.19^{a} ±0.04	1.02^{aA} ±0.04	1.28^{aA} ±0.05	1.16^{aA} ±0.05	0.83^{b} ±0.06	0.99^{aA} ±0.06	1.18^{aA} ±0.06	1.39^{aA} ±0.07

GI: Control group, GII: Acid silage, GIII: Surimi waste. In columns, means bearing same superscript (^{a}, ^{b}) do not differ significantly at 5% level. In rows, means within groups were compared between subsequent weeks and those bearing (^{A}) differ significantly at 5% level

Table 3: Effect of dietary supplementation of processed fish waste on blood lipids (mg/dl) profile of broiler chicken (mean ± SE, n = 15)

Group	Age (wks)				Age (wks)				Age (wks)				Age (wks)				Age (wks)			
	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7
	Total lipids				Triglycerides				Total cholesterol				HDL cholesterol				VLDL-Cholesterol			
GI	375.80^{a} ±25.84	491.33^{aA} ±31.61	622.67^{aA} ±28.54	725.13^{aA} ±24.99	60.13 ±3.40	68.60 ±2.75	75.00 ±2.71	85.07 ±3.27	70.93^{a} ±5.22	76.33^{aA} ±4.62	85.47^{aA} ±4.13	96.27a^{A} ±5.30	32.60^{a} ±2.28	40.80^{aA} ±1.65	47.13^{aA} ±3.00	62.87^{aA} ±2.02	12.03^{a} ±0.68	13.72^{aA} ±0.55	15.01^{a} ±0.54	16.95^{aA} ±0.67
GII	407.13^{a} ±38.55	509.73^{aA} ±28.27	622.07^{aA} ±39.23	710.87^{aA} ±38.65	64.20^{a} ±3.30	69.00^{aA} ±3.29	73.00^{aA} ±3.09	88.07^{aA} ±4.49	75.53^{a} ±3.84	82.73^{aA} ±4.21	92.80 ±4.52	103.73 ±6.92	32.47^{a} ±2.09	39.33^{aA} ±2.84	47.27^{aA} ±2.85	54.27^{bA} ±2.30	12.85^{a} ±0.67	13.80^{aA} ±0.66	14.60^{a} ±0.62	17.60^{aA} ±0.90
GIII	416.13^{a} ±38.79	525.87^{aA} ±43.38	633.60^{aA} ±49.88	733.53^{aA} ±31.66	56.13^{a} ±4.02	60.27^{a} ±2.88	66.37^{aA} ±3.06	77.13^{aA} ±2.50	67.87^{aA} ±4.30	78.53^{aA} ±5.12	87.13^{aA} ±5.21	99.00a^{A} ±5.21	31.00^{a} ±2.28	38.87^{aA} ±2.76	46.47^{aA} ±2.87	58.00^{abA} ±1.84	11.23^{a} ±0.80	12.05^{a} ±0.58	13.35^{aA} ±0.61	15.43^{aA} ±0.50

GI: Control group, GII: Acid silage, GII: Surimi waste. In columns, means bearing same superscript (^{a}, ^{b}) do not differ significantly at 5% level. In rows, means within groups were compared between subsequent weeks and those bearing (^{A}) differ significantly at 5% level

Table 4: Effect of dietary supplementation of processed fish waste on antioxidant status of broiler chicken (mean ± SE, n = 15)

Group	Age (wks)				Age (wks)				Age (wks)				Age (wks)			
	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7
	SOD (U/gHb)				Catalase (k/gHb)				GSH (nmol/ml)				LPO (nmol/ml)			
GI	234.47^{a} ±8.24	260.33^{aA} ±8.90	278.93^{aA} ±9.76	290.80^{aA} ±7.44	11.60^{a} ±0.77	14.68^{aA} ±0.95	17.04^{a} ±1.11	22.44^{aA} ±1.38	54.12^{a} ±0.42	62.25^{aA} ±0.32	80.12a^{A} ±0.60	128.92a^{A} ±0.32	0.81^{a} ±0.05	0.90^{aA} ±0.04	1.01^{aA} ±0.04	1.11^{aA} ±0.05
GII	238.07^{a} ±9.14	251.27^{aA} ±8.06	273.73^{aA} ±8.62	291.20^{aA} ±6.97	9.88^{a} ±0.44	11.96^{a} ±0.45	16.06^{aA} ±0.76	20.96^{aA} ±1.12	52.12^{a} ±0.18	59.08^{aA} ±0.53	77.60^{aA} ±0.37	127.60^{aA} ±0.33	0.70^{a} ±0.60	0.80^{aA} ±0.06	0.94^{aA} ±0.06	1.04^{aA} ±0.06
GIII	237.13^{a} ±7.81	263.00^{aA} ±8.11	281.13^{aA} ±8.33	287.00^{a} ±5.83	9.74^{a} ±0.41	11.94^{aA} ±0.38	14.52^{aA} ±0.72	18.70^{aA} ±0.99	53.20^{a} ±0.20	64.80^{aA} ±0.54	80.80^{aA} ±0.56	129.60^{aA} ±0.26	0.70^{a} ±0.06	0.75^{aA} ±0.06	0.84^{aA} ±0.05	0.92^{aA} ±0.06

GI: Control group, GII: Acid silage, GII: Surimi waste. In columns, means bearing same superscript (^{a}, ^{b}) do not differ significantly at 5% level. In rows, means within groups were compared between subsequent weeks and those bearing (^{A}) differ significantly at 5% level

Table 5: Effect of dietary supplementation of processed fish waste on blood mineral of broiler chicken (mean ± SE, n = 15)

Group	Age (wks)				Age (wks)				Age (wks)				Age (wks)				Age (wks)			
	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7	4	5	6	7
	Na (mmol/l)				K (mmol/l)				Ca (mmol/l)				Mg (mmol/l)				Fe (µmol/l)			
GI	117.12^{a} ±5.84	123.30^{aA} ±6.24	129.11^{a} ±6.04	138.78^{aA} ±6.70	3.11^{a} ±0.16	3.24^{aA} ±0.05	3.33^{a} ±0.05	3.63^{a} ±0.16	1.44^{a} ±0.05	1.63^{aA} ±0.06	1.75^{aA} ±0.06	1.97^{aA} ±0.05	0.81^{a} ±0.02	0.82^{a} ±0.08	0.97^{aA} ±0.03	1.16^{aA} ±0.05	29.36^{a} ±0.74	32.10^{aA} ±1.01	33.53^{a} ±0.95	36.52^{aA} ±1.18
GII	118.97^{a} ±7.33	119.20^{a} ±6.59	129.96^{aA} ±6.72	139.12^{aA} ±6.81	3.17^{a} ±0.07	3.17^{a} ±0.06	3.34^{aA} ±0.07	3.44^{aA} ±0.05	1.45^{a} ±0.03	1.61^{a} ±0.05	1.81^{aA} ±0.06	2.02^{aA} ±0.05	0.72^{a} ±0.01	0.90^{bA} ±0.01	0.88b ±0.02	1.02^{aA} ±0.05	29.95^{a} ±0.96	32.70^{aA} ±0.96	34.25^{aA} ±0.97	35.92^{aA} ±1.30
GIII	123.03^{a} ±9.17	126.64^{a} ±6.80	130.67^{a} ±6.50	144.45^{aA} ±6.23	3.26^{a} ±0.04	3.37^{a} ±0.06	3.39^{a} ±0.06	3.51^{aA} ±0.04	1.45^{a} ±0.04	1.60^{aA} ±0.04	1.79^{aA} ±0.08	1.93^{aA} ±0.07	0.79^{a} ±0.03	0.85^{ab} ±0.02	0.84^{b} ±0.02	1.07^{aA} ±0.04	31.39^{a} ±1.10	32.22^{a} ±1.05	34.25^{aA} ±1.21	36.36^{aA} ±1.18

GI: Control group, GII: Acid silage, GII: Surimi waste. In columns, means bearing same superscript (^{a}, ^{b}) do not differ significantly at 5% level. In rows, means within groups were compared between subsequent weeks and those bearing (^{A}) differ significantly at 5% level

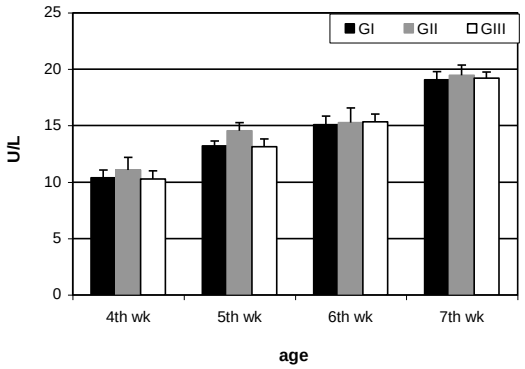

Fig. 1: Effect of dietary supplementation of fish wastes on serum ALT level in chicken (n=15). Gl: Control group, Gll: Acid silage group, and Glll: Surimi waste group

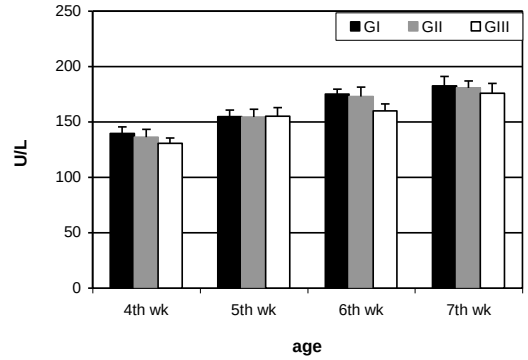

Fig. 2: Effect of dietary supplementation of fish wastes on serum AST level in chicken (n=15). Gl: Control group, Gll: Acid silage group, and Glll: Surimi waste group

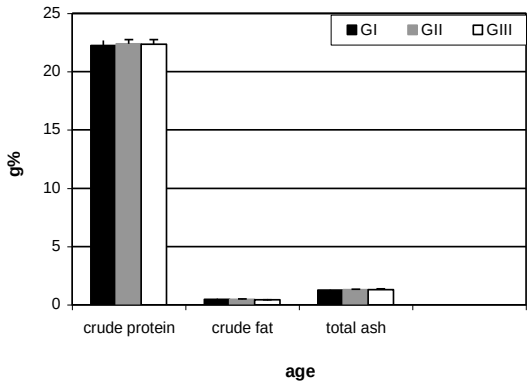

Fig. 3: Effect of dietary supplementation of fish wastes on crude protein, crude fat and total ash of chicken meat (n=3). Gl: Control group, Gll: Acid silage group, and Glll: Surimi waste group

fish wastes are a good and less expensive source of animal protein.

The levels of serum proteins and albumin were similar in all of the groups and were in agreement with those reported in broiler chicken (Emadi *et al*., 2007; Yohannan, 2007). The increase in concentration with the increase of age was similar to that observed in broiler chicken by Peebles *et al*. (1997a). The globulin level was within the normal range reported by Nworgu *et al*. (2007) and it too increased with the advancement of age. The supplemented processed fish waste was adequate enough to meet the normal protein requirements of broiler chicken.

The serum total lipids were within the normal range of broilers (Reddy, 2003). Though Yohannan (2007) observed a fluctuating trend in total lipids with the advancement of age, this increase was steady in the present study. Smitha (2005) reported that 100 percent replacement of unsalted dried fish with fermented fish waste silage on a protein basis reduced the triglyceride level of broiler chicken. According to Castillo *et al*. (1999), fish oil fed chicken expressed reduced triglycerides. The observation of no reduction in triglycerides in the present study could be due to the differences in processing and or type of fish used for the study. An age related increase in total cholesterol was similar to the findings of Dhansing (2006), and values were similar to the observations of Peebles *et al*. (1997b) and Kannan *et al*. (2005) in broiler chicken. The reduction in HDL-cholesterol towards the end of the study was similar to the reports of Castillo *et al*. (1999), where chicken supplemented with dietary fish oil expressed reduced HDL-cholesterol levels. The age related increase in HDL-cholesterol till the sixth week of age were within the normal levels observed by Yohannan (2007). Even though Castillo *et al*. (1999) observed a decrease in VLDL-cholestrol in fish oil supplemented birds, such an observation was not evident and all the groups had a similar concentration. The differences in the constituents of the fish oil and processed fish wastes could have contributed to this difference in the observations. The observations indicated that the supplemented processed fish wastes were adequate enough to meet the normal lipid metabolism.

The serum ALT and AST levels were within the normal limits as reported by Simi (2007) and Yohannan *et al*. (2007), which

indicated that the supplemented diet did not adversely affect the function of the liver and were not toxic.

Antioxidants help to stop cell destruction caused by free radicals and the body's capacity to produce antioxidants is hampered by improper nutrition. Besides, stress reduces their level in the blood and tissues. The SOD, catalase, GSH and LPO involved with the antioxidant mechanism increased with age and were similar in both the control and the treatment groups. The activities were within the normal limits observed in broiler chicken (Reddy *et al*., 2004; Ramnath *et al*., 2007), except for LPO, which expressed a lower concentration. The overall results indicated that the replacement with processed fish wastes did not hamper the normal antioxidant production and was not inducing any form of stress or strain to broiler chicken.

Minerals form the structural components of the body and are involved with the proper functioning of body systems. The major and minor elements of blood were similar in all the groups towards the end of the study and were in agreement with the concentrations observed by Bowes *et al*. (1989), Kollanoor (2004) and Francis (2005) in broiler chicken. The dietary replacement of fish meal with processed fish wastes was adequate enough to maintain the normal mineral metabolism in broiler chicken.

The level of crude protein, crude fat, total ash and cholesterol in the raw broiler meat was similar in both the control and the treatment groups. The concentration of crude protein was in agreement with the reported values of Stadelman *et al*. (1988) and Dhansing (2006). The crude fat content was lower and ash was similar to the observations of Kalavathy *et al*. (2006) and Stadelman *et al*. (1988), respectively in raw broiler chicken meat. The meat cholesterol level was within the reported values of Dhansing (2006) and Anitha *et al*. (2007) in broiler meat.

Though our previous study confirmed that these replacements lead to a reduction in the cost of poultry feed without affecting the feed efficiency and body weight of the broilers, the present results indicated that they also satisfy the nutritional requirements, have no toxicity, do not induce stress and are capable of maintaining the quality of broiler meat too. This practice would also help the fish industry to increase their income and provide a safe methodology to mitigate pollution from fish waste. Hence, the 100 percent replacement of animal protein (fish meal) in the finisher ration of broiler chicken with processed fish wastes is a management practice that could be advocated in the poultry industry. Further study could also be carried out to investigate the application of this practice in the nutritional regime of other domestic animals.

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