

**PHYSIO-BIOCHEMICAL EVALUATION OF
BROILER CHICKEN FED WITH
PROCESSED FISH WASTES**

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**Thesis submitted in partial fulfilment of the
requirement for the degree of**

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DECLARATION

I hereby declare that the thesis entitled **PHYSIO BIOCHEMICAL EVALUATION OF BROILER CHICKEN FED WITH PROCESSED FISH WASTES** is a record of research work done by me during the course of research and this thesis has not previously formed the basis for the award of any degree diploma fellowship or associateship or other similar title of any other University or Society

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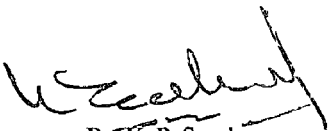


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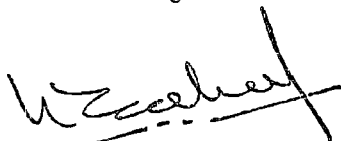
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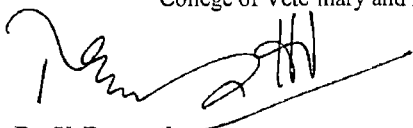
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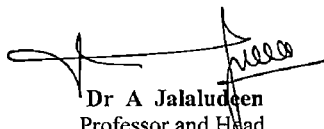
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Introduction

1 INTRODUCTION

India has made tremendous progress in broiler production in the last two decades. Chicken meat production in India has increased from 1.08 million metric tonnes in the year 2000 to 1.60 million metric tonnes during 2004 (FAO 2005). India contributes 2.39 per cent of world's total poultry meat production and is the eighth largest producer of broiler meat in the world. The poultry sector provides about 2 per cent of the total gross domestic product (GDP) of India. The per capita availability of poultry meat has increased from 600 g in 1992 to 1.3 kg in 2002 (Poultry International Executive Guide 2004).

Poultry industry in India started as a backyard activity and in the past three decades it has undergone a revolutionary change. In the coming years due to urbanization and rising income the affordability of chicken meat and eggs would increase leading to a greater demand for poultry products. In 1991 poultry accounted for 16 per cent of the total animal protein consumption and in 2000 it rose to 36 per cent. In the overall market for poultry products, India is positioned 17 in World Poultry Production.

Fish farming produces large amounts of by-products during its commercial processing. These by-products are a potential source of pollution if dumped at sea or discarded offshore. But by-products from the fishing industry and fish farming have been shown to be a valuable animal protein source in animal feed if converted into fish silage.

Fish meal is an important ingredient of broiler feed. Its protein quality as well as potency of unidentified growth factors varies greatly. Broiler diets contain 20 to 23 per cent crude protein and of this 4.5 to 5.5 per cent is constituted by animal protein, mainly fish meal. The cost of production of one kg of dried fish is

approximately Rs 15. But the cost of production of one kg of acid silage (fish waste) is Rs 4.25 while that of surimi waste powder is negligible. Since the availability of certain amino acids are limited in vegetable protein supplements, it is necessary to use animal protein sources in poultry feeds. But due to the high cost and low availability of fish meal, alternative sources of animal protein in the diet are to be explored. Instead of the usual unsalted dried fish, unconventional fish by-products are used nowadays due to high price of raw fish. In India, the processing wastes generated by many fish processing centres cause a problem of their disposal. Hence conversion of this waste into other by-products which could be included in the poultry ration is an alternative. Acid silage and surimi waste powder are two such products developed from the waste obtained during the processing of the fish Japanese thread fin bream (*Nemipterus japonicus*) by the Central Institute of Fisheries Technology (CIFT) Koch.

The present study is planned to assess whether the fish by-products namely acid silage (fish waste) and surimi waste powder could be used as alternative source of animal protein replacing dried fish/fish meal in broiler ration by investigating its physiological and biochemical parameters in broiler chicken.

Review of literature

2 REVIEW OF LITERATURE

The most commonly used organic acids for acid silage preparation were propionic, acetic and formic acids. Among these, formic acid is one of the organic acids widely used in the production of acid silage due to its property of preserving the silage and increasing the shelf life up to one year at ambient temperature of the tropical countries. A three per cent by weight of 98 per cent formic acid was added to the well ground fish mince as a preservative (Tatterson and Windsor 1974).

James *et al* (1976) at the Natural Resources Institute, London, conducted extensive feeding trials in United Kingdom and various countries in Africa and Asia and conclusively established that formic acid preserved silage is a satisfactory substitute for fish meal in poultry diet.

Kompiang *et al* (1979) worked out the chemical composition, fermentation characteristics and nutritional value of microbial fish silage. They observed that during a three week feeding period, the growth rate of the chicken fed with diets containing silage up to eight per cent was similar to that of the birds fed with standard fish meal containing rat on.

Feeding trials conducted in Thailand by Pong Pen *et al* (1979) demonstrated that growth of chicken fed on diet having five per cent silage was comparable to that of chicken fed on fish meal, the latter being marginally superior to growth of silage fed chicken.

Short term feeding trials in very young pigs in Indonesia and long term feeding trials in growing pigs in India (Rangkuti *et al* 1980, Anon 1976 and 1977) confirmed that fish silage could serve as an active protein supplement for pigs.

without any ill effects. The study conducted also revealed that feed efficiency and weight gain in pigs fed on silage at 20 per cent level in the diet were comparable to those pigs fed on fish meal (six per cent) both diets being at isoprotein level.

Raa and Gildberg (1982) observed that fish silage made by acid addition or lactic acid fermentation could be used to replace the traditional diets of soya flour or fish meal in feeding various farm animals like pigs, calves, fox, mink, sheep, beef, cattle and poultry. It was also observed that the performance of poultry broilers had been consistently poor when fed on fish silage, may be due to thiamine deficiency, use of spoiled raw materials and oxidation of fish lipids.

Johnson *et al* (1985) evaluated the nutritional quality of two types of fish silage for broiler chicken. They demonstrated that with appropriate precautions in the preparation and handling of fish before ensiling, fish silage could be incorporated into nutritionally balanced diets for broiler chicken without detrimental effects on growth or carcass taste. Live weight and live weight gain were more for birds fed with fish silage than that of birds fed with normal fish meal diet. The live weight obtained for birds fed with normal fish meal for 6 weeks was 1737 g and for birds fed with fish silage ranged from 1808 to 1896 g.

Krogdahl (1985a) used fish silage as a protein source for layer type chicks and hens. He observed that egg production and feed efficiency were not affected by diets containing 20 per cent of protein from concentrated viscera silage.

Krogdahl (1985b) evaluated fish viscera silage as a protein source for meat type chicken. He reported that all the observed characteristics of broiler performance, viz. weight gain and slaughter yield were influenced positively by fish viscera silage.

Bowes *et al* (1989) compared the serum biochemical profiles of male broilers with female broilers and white leghorn chickens. They reported that in four week and six week old normal broiler chicken the total serum protein concentration were 2.56 and 2.65 g/dl respectively, serum albumin concentration were 1.29 and 1.37 g/dl respectively and serum globulin concentration were 2.15 and 1.89 g/dl respectively. At fourth week and sixth week the AST values were 184 and 254 U/l respectively while the total serum cholesterol concentrations were 4.23 and 3.23 mmol/l respectively. There was significant difference between the control and experimental groups in the values of total protein, albumin, globulin, potassium, calcium and serum cholesterol concentrations but no significant difference was observed in the case of AST, sodium and magnesium.

Myer *et al* (1990) opined that the inclusion of scallop viscera silage at a dietary level of 24 per cent did not significantly influence average daily gain or average daily feed intake in pigs. They concluded that waste scallop viscera could be used as a high protein feed stuff in swine diets. The daily gain value obtained for zero per cent scallop viscera added ration was 0.86 kg while that of 24 per cent added was 0.87 kg. The daily feed intake values obtained for zero per cent and 24 per cent scallop viscera added ration were 2.69 and 2.66 kg respectively.

Stoner *et al* (1990) conducted two trials to evaluate a select menhaden fish meal (SMFM) as a protein source in starter diets for three week old weaned pigs. The diet containing 10 per cent dried whey and 4 per cent SMFM resulted in growth and performance similar to that from the diet containing 20 per cent dried whey and zero per cent SMFM. They indicated that 4 per cent SMFM could replace half the dried whey in a 20 per cent dried whey starter diet and concluded that menhaden fish

meal could be used as the major protein source in starter diet for pigs. The daily feed intake of pigs of zero to two weeks fed 4 per cent SMFM and 10 per cent dried whey was 290 g and of those fed zero per cent SMFM and 20 per cent dried whey was 289 g.

The effect of feeding sardine fish oil on the metabolism of lipoproteins was studied in rats by Anil *et al* (1992). Rats fed diets containing 10 per cent sardine expressed a significant decrease in the total cholesterol, triglycerides, VLDL in serum in fish oil fed rats. They concluded that the activity of lipoprotein lipase in adipose tissue and aorta was significantly higher in rats fed sardine oil which caused an increased clearance of triglyceride rich lipoproteins from circulation.

Espe *et al* (1992) substituted fish silage protein for fish meal protein in White Leghorn chicken. They observed that chicken fed with fish silages in graded amounts showed the same or better weight gains and feed efficiencies compared with chicken fed with dietary fish protein as fish meal.

Turtock (1992) carried out trials to investigate the potential of small pelagic fish (*Rastriobola argentinus*) locally referred to as omena as a protein supplement for pigs. The control group was given soyabean meal. Average daily gains and daily feed intakes were similar between the two dietary treatments and concluded that *Rastriobola argentinus* is a potential protein supplement for pigs.

Connor *et al* (1993) studied the effects of n-3 fatty acids from fish oil on plasma lipoproteins and hypertriglyceridemic patients. They found that dietary n-3 fatty acids from fish and fish oil had profound hypolipidemic effects in normal subjects and in hypertriglyceridemic patients with hyperlipidemia. In metabolic experiments, reductions occurred in plasma triglycerides, plasma total cholesterol

very low density lipoproteins, chylomicrons and low density lipoproteins. They opined that the use of fish oil supplements could be regarded as a pharmacologic therapy particularly effective in severe hypertriglyceridemic states and that a life long diet rich in fish might be protective against atherosclerosis. The hypolipidemic effects of n-3 fatty acids coupled with the r ant thrombotic actions appeared to have an important potential role in the control of coronary heart disease and other atherosclerotic disorders.

Fagbenro *et al* (1994) used dry diets containing varying levels of dried fermented fish silage and soya bean blend as replacement for 25, 50 or 75 per cent fish meal protein in aquaculture feeds. There was no significant difference in the final body weight between control and treatment groups. Results showed that a well balanced dry feed based on co-dried fermented silage was as efficient in supporting growth as a dry fish meal based diet and that it represented an alternative to fish meal in utilizing waste or trash fish, under sized or low value fish as protein feed stuff for aquaculture species.

Rose *et al* (1994) compared the nutritional value of naturally fermented fish silage with formic acid fermented fish silage in pigs. They found out that the feed intake of the pigs given the naturally fermented silage were 12 per cent greater than those given the acid silage. Poor flavour characteristics of the fish silage probably reduced the voluntary feed intake of the pigs and resulted in poor productive performance.

Fagbenro and Jauncey (1995) studied the growth and protein utilization by juvenile catfish fed with fish silage. Fish silage prepared from lactic acid fermentation of whole tilapia, 5% sugar beet molasses and 2% *Lactobacillus plantarum* were used. They found that the haematocrit and haemoglobin contents

showed no significant differences among the silage treatments. They obtained mean values for PCV and Hb as 30.8% and 8.6 g/100 ml respectively. They concluded that fermented fish – silage co-fermented with protein feedstuffs was a suitable protein supplement without affecting feed efficiency, growth or health.

Prawn processing wastes upon ensilation contained value added nutrients like carotenoid pigments and n-3 polyunsaturated fatty acids which are released by ensilation but are otherwise difficult to extract even by solvents (Guillou *et al* 1995).

Kasat and Baghel (1995) studied the protein utilization in broilers offered ten isocaloric (2900 K Cal ME/kg) and isonitrogenous (23 per cent CP) diets containing different levels (2.2, 5.3, 5.4, 5.5, 5.6 and 6.5%) of fish meal protein. They concluded that most efficient protein utilization was observed in broilers offered 23 per cent CP diet containing 6 per cent fish meal protein along with vegetable protein supplement.

Rosenfeld *et al* (1997) conducted a study to measure the effect of substituting different levels of shrimp meal for soyabean meal in broiler diets. Study revealed that carcass weight increased significantly by 12.1% when shrimp meal was substituted 100% for soyabean meal. They concluded that shrimp meal used could partially or totally replace soyabean meal in broiler diets without negatively affecting performance or carcass quality.

Peebles *et al* (1997a) studied the effects of addition of lard to the starter diets in broiler chicken up to six weeks of age. They observed that as the age advanced the body weight and haematocrit values of the birds increased and also body weight of male chicken were normally more than female chicken. The VPRC of fourth week

and sixth week old normal broiler chicken were 26.50 % and 30.40 % and was similar to experimental groups. According to their study, the total plasma protein concentration in normal broiler chicken of four, five and six weeks of age were 3.96, 4.05 and 4.08 g/dl respectively, which was similar to that of the experimental groups.

A study was conducted by Peebles *et al* (1997b) to assess the effects of addition of lard to starter diets in broiler chicken up to six weeks of age. They found that in normal broiler chicken from fourth to sixth week of age, the concentration of triglycerides ranged from 103 to 164 mg/dl. The serum HDL concentration ranged from 78 to 88 mg/dl, which was significantly different from the experimental groups. VLDL concentration ranged from 21 to 33 mg/dl and total serum cholesterol concentration ranged from 115 to 122 mg/dl and all the values were significantly different from the control groups.

Razdan *et al* (1997) studied the response of feeding chitosan and pectin in two week old broiler chicken. They reported that in the plasma of two week old normal broiler chicken, the triglyceride, HDL and total cholesterol concentration were 0.34, 2.89 and 7.78 mmol/l respectively. There was no significant difference between control and experimental groups in triglyceride and HDL concentrations but total cholesterol showed a significant difference.

Palod and Baghel (1998) undertook a study to find the effect of feeding varying levels of fish meal protein (FMP) on carcass traits of broilers. They observed that 5 per cent FMP along with soyabean meal was best for efficient carcass yield. They also commented that the mean value of giblet weight for the 5 % group was 51.24 g.

Hammoumi *et al* (1998) conducted feeding trials in broilers using fermented fish waste. The nutritional assays showed a net increase in the broiler weight relatively to the control diet.

An investigation was conducted by Kanagaraju (1998) to assess the influence of phytase on phosphorous utilization in broilers. The mean body weight obtained for normal broiler chicken of four to eight weeks of age ranged from 703 to 1480 g and the mean values of g/bled weight of normal broiler chicken of eight weeks of age was 123 g. They also reported that the mean serum Ca concentration at sixth week of age was 11.03 mg%. According to the study there was no significant difference between the control and experimental group in the case of body weight but there were significant differences in case of g/bled weight and serum Ca.

Stadelman *et al* (1988) reported that in raw broiler chicken meat the protein content was 21.39 g, the fat content was 1.65 g and the ash content was 0.98 g per 100 g edible portion. They also observed that the cholesterol content of poultry meat was 75 mg per 100 g edible portion.

Castillo *et al* (1999) investigated the effect of dietary fish oil in the cholesterol and arachidonic acid levels in chick plasma and VLDL. The study showed that supplementation of 10 per cent menhaden oil to the chick diet for seven days produced a significant hypocholesterolemia and hypotriglyceridemia. Total cholesterol and triacylglycerol contents decreased in HDL. All chemical constituents of VLDL significantly decreased after the first week of menhaden oil supplementation to the diet.

Imaeda (1999) characterized serum enzyme activities and electrolyte levels in 6 week old broiler chickens and noted that the mean Na and K values of the normal

control birds were 158 ± 4 and 58 ± 0.5 mmol/l respectively. The mean SGOT value was 128 ± 3 U/l.

Kjos *et al* (1999) studied the effects of dietary fish silage and fish fat on growth performance and carcass characteristics of growing and finishing pigs. They found no negative effects on growth performance and carcass quality.

Vizcarra Magana *et al* (1999) conducted a study on the nutritional evaluation of silage prepared from tuna fish wastes in broilers and found that the dried product obtained could be successfully incorporated in broiler diets at levels up to 15 per cent without adverse effects. The average weight gain on using 0, 5, 10 and 15 % silage in the diet were 519.83 ± 16.43 , 541.7 ± 6.8 , 517.2 ± 16 and 499.3 ± 15.5 g and the average feed intake were 757 ± 163 , 809 ± 19.26 , 749 ± 32.3 and 794 ± 13.05 g respectively.

Balasubramanian (2000) studied the influence of microbial phytase on nutrient utilization in broiler chicken. The mean body weight obtained for normal broiler chicken of four to eight weeks of age ranged from 1030 to 1832g and the mean giblet weight of chicken of six week and eight week of age were 111.94 and 159.58g respectively. The mean serum Ca concentration at sixth week of age was 12.03 mg% according to the study. All the values were statistically comparable to those of the experimental groups.

Protein quality of shrimp waste meal (SWM) was assessed in a balanced experiment with 30 rats (Fanimo *et al* 2000). The rats were fed four different 10 per cent protein diets (consisting of) namely fish meal, SWM, SWM + lysine + methionine, SWM + methionine and a nitrogen free basal diet. Shrimp waste meal reduced the relative weights of lungs, liver and intestines. Rats fed with SWM diet

had lower plasma protein and albumin. Plasma Na and K were increased with amino acid supplementation of SWM diet. The results showed that the protein quality of SWM was inferior to that of fish meal but the supplemented amino acids in SWM diets improved the quality of the protein.

Puvadolpirod and Thaxton (2000) studied the effect of stress in 5 week old chicken. They observed that the mean values of RBC count and WBC count of normal control group were 2.01 ± 0.08 ($\times 10^6/\mu\text{l}$) and 11.31 ± 0.09 ($\times 10^3/\mu\text{l}$) respectively which were similar to the experimental groups. They also found that the mean values of plasma cholesterol, triglycerides and HDL for the normal control group were 87.38 ± 3.85 , 94.88 ± 14.44 and 63.50 ± 3.09 mg/dl respectively which were lower than the values in the experimental groups.

Anil (2001) conducted a study to assess the effect of sodium sulphate supplementation in broiler diet and mean values obtained in normal broiler chicken for the total serum protein concentration at eighth week of age was 4.97 g %. The giblet weight of normal birds at eight weeks of age was 159 g. The mean body weight at fourth and sixth weeks of age ranged from 1012 to 1840 g. There was no significant difference in the values between control and experimental groups.

Kadari (2001) investigated the effect of probiotic supplementation on the performance of broiler chicken up to eight weeks of age. The body weights of normal broiler chicken from fourth to eighth weeks of age ranged from 924 to 2092 g. In eight week old normal broiler chicken the serum protein concentration was 4.16 g/dl. The giblet weight of eight week old broiler chicken was 73.43 g. The growth of vital organs depends on the nutritional status of the birds (Bhosale and Rao 2001). All these values were statistically comparable with that of experimental groups.

Kjos *et al* (2001) studied the effects of dietary fish silage and fish fat on the performance and egg quality of laying hens. They observed that fish by products preserved as concentrated defatted fish silage could be fed as a part of the compound feed for laying hens. In the study an inclusion level of 50 g/kg diet supplementing 12 per cent of the total protein had no effect on egg production.

Ngoan *et al* (2001) conducted feeding trials to evaluate the effects of replacing fish meal (FM) with ensiled shrimp by product (ESB) in a cassava root meal and rice bran based diet on the performance and carcass characteristics of growing pigs. The crude protein of the FM was replaced with 0, 50 or 100 per cent ESB. The study revealed that animal growth performance and daily feed intake were significantly reduced by the inclusion of shrimp by products in the diets whereas carcass measurements were not significantly affected. Daily weight gain of the pigs fed with 100 per cent FM diet and 50 per cent ESB diet were significantly higher than those fed with 100 per cent ESB diet. They concluded that from economical as well as performance point of view ensiled shrimp by product could replace 50 per cent of the crude protein of fish meal for growing pigs.

Newman *et al* (2002) studied the effects of dietary n 3 and n 6 fatty acids on chicken metabolism and found that the mean value of NEFA was 200 $\mu\text{mol/l}$ for the normal control group fed with fish oil which was not significantly different from the experimental groups fed with sunflower oil and tallow.

Sands and Smith (2002) studied the effects of dietary manganese proteinate and chromium picolinate supplementation on NEFA in broiler chickens reared under thermoneutral conditions. They found that in normal control group of birds the NEFA concentration was 520 $\mu\text{mol/l}$ which was similar to that of chromium

picolinate supplemented group and was significantly higher than manganese proteinate supplemented group

The effects of copper sulphate on meat cholesterol of broiler chicken were investigated by Skirvan *et al* (2002) They observed that in six weeks old normal broiler chicken the meat cholesterol was 62.50 mg/100g and was significantly higher than the experimental groups

Stephenson (2002) observed that Hb, VPRC, TEC and TLC reflect the overall health status of an animal. Ikekwumere and Herbert (2003) assessed the physiological responses of four week old broiler chickens to quantitative water restrictions. They noted that the mean values of VPRC, Hb and serum ALT in normal control group of birds were 38 %, 13 g % and 22.10 U/l respectively.

Ashwell and McMurty (2003) conducted studies to determine if metformin possess hypoglycemic and anorectic effects in broiler chicken. They reported that the concentration of NEFA in four week old normal broiler chicken was 206 $\mu\text{mol/l}$. A significant difference was not noted between the control and experimental groups.

Maigalema and Gernat (2003) measured the effect of substituting elevated levels of tilapia by product meal for soyabean meal in broiler diets. The results showed that chicks fed upto 50 per cent level tilapia by product meal had significantly higher body weights, carcass weights, feed consumption and improved feed conversion as compared to those fed with soyabean meal.

Reddy (2003) conducted a study on the antioxidant and hypolipidemic effects of spirulina and natural carotenoids in broiler chicken. In four, six and eight week

old normal broiler chicken the mean VPRC values were 29.75 % 31.63 % and 29.63 % respectively while the mean RBC count were 3.04 3.09 and 2.9 ($\times 10^6 / \mu\text{l}$) The mean WBC count from fourth to eighth week ranged from 23.74 to 26.49 ($\times 10^3 / \mu\text{l}$) There was no significant difference in the values between the groups

Islam *et al* (2004) conducted an experiment to study the haematological parameters in Fayoumi Assil and local chicken of different ages reared in Bangladesh They found out that erythrocyte numbers haemoglobin concentration and packed cell volume increased with the advancement of age in all the three breeds They observed that the mean values of haemoglobin VPRC and total erythrocyte count of local chicken of one month of age were $7.73 \pm 0.14 \text{ g \%}$ $27.73 \pm 1.21 \%$ and $1.70 \pm 0.04 (\times 10^6 / \mu\text{l})$ and there was no significant difference in these values with that of the Fayoumi and Assil breeds

An investigation over a period of eight weeks was carried out by Kollanoor (2004) to study the effect of dietary iron and supplementation of phytase on growth and mineral availability of broiler chicken The serum Ca Mg and Fe levels in six week old normal chicken were 11.89 mg % 1.79 mg % and 58.17 $\mu\text{mol/l}$ respectively and the values were similar to the experimental groups

The effect of heat stress on production parameters and immune responses of commercial laying hens was investigated by Mashaly *et al* (2004) They observed that the WBC count of normal laying hens increased as the age advanced and reported that the WBC count at 31 and 34 weeks of age were 3.50 and 5.50 ($\times 10^3 / \mu\text{l}$) which was significantly different from the experimental groups

Reddy *et al* (2004) studied the antioxidant and hypolipidemic effects of spirulina and natural carotenoids in broiler chicken They observed that the mean

values of blood super oxide dismutase catalase reduced glutathione and lipid peroxidation for control group of brds were 260.36 ± 77.63 U/g Hb 8.00 ± 1.34 k/g Hb 50.7 ± 2.41 nmol/ml and 3.65 ± 0.17 nmol/ml respectively and were significantly different from the experimental groups. They observed that the mean values of total lipids and triglycerides of normal control broiler chicken were 543.07 ± 76.98 mg/dl and 97.28 ± 9.21 mg/dl respectively and were significantly different from the experimental groups.

An investigation was conducted by Renjith (2004) to study the effect of dietary supplementation of baker's yeast in broiler chicken up to eight weeks of age. The mean body weight of the normal broiler chicken of fourth, sixth and eighth weeks of age was 1118, 1974 and 2584 g respectively. The values were statistically similar except for the fourth week in which the body weight of the control group was significantly lower than that of experimental groups. The mean giblet weight of eight week old normal broiler chicken was 96.38 g which was similar to the experimental groups. The normal broiler chicken of eight weeks of age had a total serum protein concentration of 3.3 g/dl and was similar to the experimental groups.

Bunchasak *et al* (2005) investigated the effect of dietary protein on immune responses of laying hens during peak production period. They observed that the mean values of albumin, globulin and albumin globulin ratio were 1.14 ± 0.16 g/dl 2.20 ± 0.40 g/dl and 0.60 ± 0.26 respectively in control group. There was no significant difference in these mean values from the experimental groups.

Dong *et al* (2005) studied the effect of replacing fish meal with ensiled shrimp waste on the performance of growing cross bred ducks. They found out that gizzard weight increased with increasing intakes of ensiled shrimp waste. They concluded that replacing around 20 per cent of the fish meal in the diet with ensiled

shrimp waste reduced the feed cost without negatively affecting the growth performance

An investigation over a period of six weeks was carried out by Francis (2005) to study the effect of dietary cation anion balance on growth performance of broiler chicken. In six weeks old normal broiler chicken serum cholesterol and Calcium concentration were 149 and 6.49 mg/dl respectively while the serum Sodium and Potassium concentration were 115.44 and 2.40 mmol/l respectively. The observations were similar with that of the experimental groups.

An experiment was conducted by Govindan (2005) to assess the utilization of dried cuttle fish waste (CFWS) on replacement of dried fish (DF) in indigenous layer duck ration. Three groups were fed with dietary combinations of 10 per cent DF and zero per cent dried CFWS, 5 per cent DF and 11.45 per cent dried CFWS and 22.9 per cent dried CFWS replacing DF completely and the diets were made isocaloric and isonitrogenous. The overall mean daily feed consumption was statistically comparable between various treatment groups. There was an increase in daily feed intake in all the groups as the age advanced.

A study was conducted by Kannan *et al* (2005) to understand the influence of probiotics supplementation on lipid profile of broilers. They reported that the control group obtained the values for total cholesterol, triglycerides, HDL and VLDL as 196.77 mg/dl, 82.52 mg/dl, 111.31 mg/dl and 16.50 mg/dl respectively. The mean values of total cholesterol and triglycerides were significantly higher than the experimental groups while the VLDL concentrations were similar.

Kroliczewska and Zawadzki (2005) conducted an experiment to study the influence of skullcap root addition on calcium, magnesium and iron levels in broiler

chicken serum till 6 weeks of age. They found that the mean values of calcium, magnesium and iron for the control group of birds at 6 weeks of age were 1.94 ± 0.13 mmol/l, 0.71 ± 0.27 mmol/l and 18.63 ± 1.94 μ mol/l respectively. The Mg concentration was similar in the control and experimental groups while the Ca and Fe concentrations were significantly different between the control and experimental groups.

Lekshmy (2005) studied the utilization of dried cuttle fish waste silage in Japanese quail layer ration and reported non significant effect in the body weight of 26 week old birds when fish meal was replaced by fish waste silage.

Moniello *et al* (2005) studied the effect of age on the metabolic profile of ostriches. They observed that the serum K, Ca and Fe concentration increased but Na and Mg concentration decreased with the advancement of age.

Ojewola *et al* (2005) investigated the effect of including three unconventional animal protein sources in broiler ration for a 49 day experiment. Locally processed fish waste meal, cray fish waste meal and grasshopper meal were compared with control diet without any animal protein. The replacement did not negatively affect the giblet weight, an indication that satisfactory animal protein could be prepared and utilized from these unconventional sources. The final body weight of the birds ranged from 1510 to 1877.8 g. The mean giblet weight was 88.25 g for locally processed fish waste meal. The values obtained by using locally processed fish waste meal for crude protein, ether extract and ash were 58.6, 9.63, 4 and 87.3 g/kg DM respectively.

Smitha (2005) conducted an investigation over a period of six weeks to study the effect of replacing unsalted dried fish with fermented fish waste silage on

nutrient utilization and growth performance in broiler chicken. The three groups of birds were maintained on isonitrogenous and isocaloric rations in which protein of unsalted dried fish was replaced with protein from fermented fish waste silage at zero, 50 and 100 per cent levels. The serum triglyceride concentration for zero, 50 and 100 per cent inclusion levels were 124, 94 and 87 mg/dl respectively which were significantly different. The serum Ca concentration for zero, 50 and 100 per cent inclusion levels were 9.70, 10.40 and 9.40 mg/dl respectively which were not significantly different.

Dhansing (2006) studied the effect of garlic powder and neem seed cake in broiler chicken. The total cholesterol concentration in fourth and sixth week old normal broiler chicken were 161 and 182 mg/dl respectively. The meat cholesterol and meat crude protein values for six week old normal broiler chicken were 54 mg/dl and 21.99 g % respectively. There was no significant difference between control and experimental groups in the meat crude protein value but a significant difference was observed in serum and meat cholesterol values.

An experiment was conducted by Kalavathy *et al* (2006) to study the effects of *Lactobacillus* feed supplementation on cholesterol, fat content of the liver, muscle and carcass of broiler chicken. They reported that the meat cholesterol and ether extract in six week old normal broiler chicken were 60 mg/100g and 0.89 per cent. The ether extract was significantly higher than that of experimental group but the cholesterol value was similar to that of the experimental group.

An investigation was conducted by Talebali and Farzimpour (2006) to study the effects of different levels of perlite on performance of broiler chicks. The feed consumption was found to increase with the advancement of age in all the groups of treatment.

Adeyemo and Longe (2007) studied the effects of cottonseed cake on broiler chicken up to eight weeks of age and reported that the weekly feed consumption from fifth to seventh weeks of age ranged from 819 to 899 g. A significant difference was observed only on seventh week between control and experimental groups. The total serum protein in normal broiler chicken at eight weeks of age was 11.93 g/dl which was significantly different from the experimental groups.

An experiment was conducted by Anitha *et al* (2007) to study the inclusion of crude rice bran oil on production performance, carcass characteristics and biochemical parameters in broiler chicken for a period of seven weeks. They observed that the concentration of total serum cholesterol and meat cholesterol in normal broiler chicken of seven weeks of age were 91 and 86.79 mg/dl. The values were similar to that of the experimental groups. In seven-week-old normal broilers, the triglyceride and HDL concentrations were 12.35 and 26 mg/dl respectively. The observed values were similar to that of the experimental groups.

Barroga *et al* (2007) investigated the effect of fish silage mixed diets on growth performance and carcass characteristics of fattened paddy herded ducks and concluded that the ducks could adapt well to fish silage mixed diets without adverse effects on their growth performance and carcass quality.

The effect of turmeric rhizome powder on blood parameters of broiler chicken up to six weeks of age was investigated by Emadi *et al* (2007). They found that in five-week and six-week-old normal broiler chicken, the serum total protein concentration were 4.50 and 4.20 mg/dl respectively, the serum albumin concentration were 1.50 and 1.52 g/dl respectively, and the serum globulin concentration were 3.00 and 2.72 g/dl respectively. They reported that at fifth and

sixth weeks of age the serum triglyceride concentrations were 106.60 and 102.40 mg/dl respectively the serum HDL concentration were 165 and 147 mg/dl respectively The serum VLDL concentrations were 23.6 and 20.8 mg/dl respectively and the serum total cholesterol concentrations were 132 and 122 mg/dl respectively There was no significant difference between the control and experimental groups in the globulin and triglyceride concentrations while albumin HDL VLDL and cholesterol concentrations were significantly different

Fasuyi (2007) studied the effect of *Amaranthus cruentus* leaf meal as a protein supplement in broiler finisher diets and reported that the body weight of normal broiler chicken of fourth and sixth weeks of age were 787 and 1260 g respectively which were similar to those of experimental groups An age related increase in body weight was also observed

Maini *et al* (2007) conducted a comparative study of antioxidants in broilers They observed that in normal broiler chicken of three and five week of age the GSH levels were 1.33 and 0.97 mmol/l respectively while the LPO levels were 12.44 and 9.24 nmol/ml respectively They reported that the SOD levels at third and fifth weeks of age were 59 and 56 U/mg Hb respectively All the values were significantly different from the experimental groups

Nworgu *et al* (2007) carried out an experiment to evaluate the performance and blood chemistry indices of broiler served fluted pumpkin leaves extract supplement They found that the mean haemoglobin and PCV values of normal control group were 7.66 g % and 28 % respectively They also observed that the mean values of albumin globulin A G cholesterol Na and K values were 2.10 g /dl 1.4 g/dl 1.5 143 mg/dl 103.1 nmol/l and 4.4 nmol/l respectively The

mean values of the control group were significantly lower than that of the experimental groups

An experiment was conducted by Raghavan (2007) in Japanese quail layers from 7 to 26 weeks of age by 100 per cent replacement of unsalted dried fish with dried fish waste and fermented fish waste silage on protein basis. The body weight during observation period for different dietary treatments did not differ significantly. The overall evaluation of the study revealed that dried fish waste and fermented fish waste silage could be used economically to replace unsalted dried fish protein completely in Japanese quail layer rations without any adverse effect on overall performance.

Ramnath *et al* (2007) conducted a study on four week old local strain male chickens to investigate the effect of an ayurvedic supplement on heat stressed chickens on certain haematological and biochemical variables. They found that the mean values of GSH, blood catalase, SOD and serum lipid peroxidation level were 144.88 nmol/ml, 24.04 k/g Hb, 294.63 U/g Hb and 2.39 nmol of MDA formed /ml respectively for the control group of birds. LPO value in control group was significantly lower than that of experimental groups. Blood catalase, GSH and SOD values were significantly different in the control and experimental groups.

An experiment was conducted by Simi (2007) to study the effect of dietary supplementation of turmeric on the performance of broiler chicken for six weeks. The mean values for Hb, VPRC, TEC and TLC in six weeks old normal broiler chicken were 8.35 g%, 26.88 %, $2.97 \times 10^6/\mu\text{l}$ and $22.16 \times 10^3/\mu\text{l}$ respectively. The values of Hb, VPRC and WBC count in control group were significantly different from the experimental group but the RBC count was similar in all the treatment groups. The ALT and AST values at sixth week of age were 178 and 6.50 U/l.

respectively which was significantly different from the values of experimental groups. In six week old broiler chicken the serum total lipid and total cholesterol concentrations were 714 and 176 mg/dl respectively and they were significantly higher than the rest of the groups.

Yohannan (2007) conducted a study on the physiological evaluation of dietary supplementation of steroid hormones and alpha tocopherol in broiler chicken up to eight weeks of age. There was an increase in the TEC from fourth to eighth week of age in all the treatment groups. The albumin concentration in normal chicken from four to eight week ranged from 1.87 to 1.96 g/dl and the globulin concentration ranged from 3.01 to 3.15 g/dl and the Albumin:Globulin ratio ranged from 0.59 to 0.64. The AST value from fourth to eighth ranged from 195 to 211 U/l. In the plasma of normal broiler chicken of fourth to eighth week of age the total lipid concentration ranged from 514 to 538 mg/dl, the triglycerides concentration ranged from 108.62 to 109.73 mg/dl, the HDL concentration ranged from 41 to 52 mg/dl, the VLDL concentration ranged from 21.72 to 21.94 mg/dl and the total cholesterol concentration ranged from 129 to 136 mg/dl. The values of SOD, catalase and LPO in fourth, sixth and eighth week old normal broiler chicken ranged from 1837 to 4116 U/g Hb, 2.46 to 4.77 k/g Hb and 1.80 to 2.63 nmol/ml respectively. There was significant difference between the control group and experimental groups in the concentrations of albumin, AST, total lipids, LDL, HDL and total cholesterol at sixth week and eighth week of age. In the case of triglyceride concentration a significant difference was observed between control and experimental groups throughout the study. There was no significant difference in SOD, Catalase and LPO values between the control and experimental groups at fourth and eighth weeks of age but at sixth week of age a significant difference was observed.

Materials and methods

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

Forty five day old broiler chicks (Vencob strain) procured from Coastal Krishna Hatcheries Ollukkala were reared under standard managemental conditions in a battery brooder. They were fed with commercial broiler starter ration for the first three weeks and divided into three groups G I G II G III comprising 15 birds per group from four weeks of age. The study was carried out from fourth week to seventh week of age.

Birds in G I were fed with standard broiler finisher ration of BIS specification.

Birds in G II were fed a standard broiler finisher ration in which the unsalted dried fish was completely replaced with acid silage (fish waste) and the feed was made isocaloric and isonitrogenous with the control finisher ration.

Birds in G III were fed a standard broiler finisher ration in which the unsalted dried fish was completely replaced with surimi waste powder and the feed was made isocaloric and isonitrogenous with the control finisher ration.

The processed fish wastes acid silage (fish waste) and surimi processed waste were prepared and supplied by Central Institute of Fisheries Technology (CIFT) Kochi. Acid silage (fish waste) was prepared by mixing fish waste (*Nemipterus japonicus*) with 3 per cent (w/v) of formic acid. It was kept for 7 to 10 days with daily stirring and the dried and powdered. Surimi waste was prepared by cooking fish waste (*Nemipterus japonicus*) as such with 20 per cent (w/v) water for 30 minutes. The cooked water was then drained off and solid was dried in electrical tunnel drier.

Proximate analysis of acid silage (fish waste) and surimi waste powder are given in table 1 and the compositions of experimental diets are presented in table 2.

Table 1 Proximate analysis of acid silage (fish waste) and surimi waste powder

Parameter	Acid silage(fish waste)	Surimi waste powder
Moisture	18%	5.29%
Dry matter	82%	94.71%
Total ash	35.4%	40.19%
Acid insoluble ash	0.514%	0.341%
Crude protein	50.3%	49.54%
Crude fat	6.28%	6.55%

Table 2 Per cent ingredient composition of experimental diets

Ingredients	Group I	Group II	Group III
Maize	61.1	63.5	62.2
Deoiled rice bran	9.3	4.8	4.1
Soya bean meal	18.4	20.5	22.3
Unsalted dried fish	10		
Acid sludge (fish waste)		10	
Surm waste powder			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 per 100 kg feed			
Vitamin mixture g	10	10	10
Lysine ¹ g	100	100	100
Choline chloride ² g	120	120	120
Cocciostat ³ g	50	50	50
Toxin binder ⁴ g	250	250	250
B complex powder ⁵ g	50	50	50

Note

Vitamin mixture INDOMIX A B2 D K powder (N cholas Primal Ind a Ltd Mumba) contain ng
V t A 82 500 IU V t B₂ 50 mg V t D 1200 IU V t K 10 mg per g

L Lysine Aj nomoto Co Bangkok Thailand Ltd contain ng monohydrochloride 98.5 %

²B choline Ind an Herbs Research and Supply Co Ltd (U P) contain ng choline chlor de 50 %

³Elancoban 220 (Elanco Animal Health El L lly and Co Ind ana Pol s USA) contain ng Monens n
sodium 10 %

⁴UTPP 5 powder (Tetragon Chem c Pvt L d Banga ore) contain ng Treated Aluminosilicates
Prop onates Formates Acetates

⁵Merplex FDS (Wockhardt Ltd Wockhardt towers Bandra Kurla Complex Mumba 400 051)
contain ng V t B₈ 8 mg V t B₆ 16 mg V t B₂ 80 mg N acin 120 mg Folic acid 8 mg pantothenate
80 mg per g

3.2 ESTIMATION OF BODY WEIGHT AND FEED CONSUMPTION

The body weights of the birds were recorded from fourth week to seventh week of age at weekly intervals. The feed supplied and left over in each week were recorded from fourth week to seventh week of age and the feed consumption of the birds was then calculated.

3.4 Blood Collection

Blood samples (10ml) were collected by wing vein puncture of birds with or without anticoagulant (heparin) from G I, G II and G III from fourth week of age at weekly intervals till seventh week for haematological and biochemical tests. The blood was centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was stored at 20°C till further analysis. On the day of final blood collection, birds were sacrificed by cervical dislocation. Gizzard (heart, liver and gizzard) was excised out and thoroughly washed with water and weighed. A piece of meat (10 g) from the cranial aspect of pectoral region of each bird was excised for cholesterol estimation and proximate analysis.

3.5 ESTIMATION OF HAEMATOLOGICAL PARAMETERS

3.5.1 Haemoglobin

Haemoglobin level was determined by the standard procedure of ferritheme hydrochloride method (Sastrı, 1998).

3 5 2 Volume of Packed Red Cells (VPRC)

Volume of packed red blood cells (VPRC) was estimated by microhematocrit method

3 5 3 Total erythrocyte and leucocyte count

The method described by Natt and Herrick s (1952) was followed for total erythrocyte count and total leucocyte count

The composition of reagent used

Natt and Herrick s Fluid

Sodium Chloride	3 88 g
Potassium Chloride	2 50 g
Disodium Hydrogen	
Phosphate Dodecahydrate	1 44 g
Potassium Dihydrogen	
Phosphate	0 25 g
Formalin (37 per cent)	7 50 ml
Methyl Violet 2B	0 10 g
Distilled Water	1000 ml

The above preparation was stirred overnight filtered and used

Enumeration of RBC

1:200 dilution of blood with Natt and Herrick's reagent was done using RBC diluting pipette. After mixing and loading on haemocytometer kept for five minutes RBCs located in 4 corner and central squares were counted (A)

$$\text{Total RBCs} = A \times 10,000 / \mu\text{l of blood}$$

Enumeration of WBC

1:200 dilution of blood with Natt and Herrick's reagent was done using RBC diluting pipette. After mixing and loading on haemocytometer kept for five minutes WBCs located in the 9 large squares in the ruled area were counted (B)

$$\text{Total WBCs} = (B + 10 \%B) \times 200 / \mu\text{l of blood}$$

3.6 ESTIMATION OF BIOCHEMICAL PARAMETERS

3.6.1 Serum protein profile

3.6.1.1 Serum total protein Serum total protein was estimated by Biuret method (Henry *et al* 1957) using Ecoline[®] Kit (M/S E Merck India Limited Mumbai)

3.6.1.2 Serum albumin Serum albumin was estimated by the method of Doumas *et al* (1971) using Ecoline[®] Kit (M/S E Merck India Limited Mumbai)

3.6.1.3 Serum globulin Serum globulin content was calculated as the difference between serum total protein and albumin contents

3 6 1 4 Albumin Globulin ratio The albumin/ globulin ratio was calculated using the following formula

$$\text{Albumin Globulin ratio} = \frac{\text{Concentration of Albumin (g/dl)}}{\text{Concentration of Globulin (g/dl)}}$$

3 6 2 Serum enzymes

3 6 2 1 Serum alanine aminotransferase (ALT) The level of ALT in the serum was determined by U V kinetic method (Bergmeyer 1974) utilizing the kit supplied by Agappe Diagnostics Pvt Ltd Maharashtra

3 6 2 2 Serum aspartate amino transferase (AST) The level of AST in the serum was determined by U V kinetic method (Bergmeyer 1974) utilizing the kit supplied by Agappe Diagnostics Pvt Ltd Maharashtra

3 6 3 Serum lipid profile

3 6 3 1 Serum total lipids Concentration of serum total lipids was estimated by Phosphovamline method as described by Zoller (1962) using Labkit[®] Kit (M/S LabKit Spain)

3 6 3 2 Serum HDL cholesterol Serum HDL cholesterol was estimated by precipitation method using phosphotungstate Magnesium chloride (Bachorik *et al* 1976) using kit procured from Agappe Diagnostics Maharashtra

3 6 3 3 Serum total cholesterol The concentration of total serum cholesterol was estimated by cholesterol Phenol Aminoantipyrine (CHOD PAP) method as

suggested by Richmond (1973) using Ecoline[®] Kits (M/S E Merck India Limited Mumbai)

3 6 3 4 *Serum triglycerides* Concentration of serum triglycerides was estimated by a method suggested by Schettler and Nussel (1975) using Ecoline[®] Kits (M/S E Merck India Limited Mumbai)

3 6 3 5 *Serum VLDL* Concentration of serum VLDL cholesterol was estimated using Friedewald equation (Friedewald *et al* 1972)

VLDL

Triglycerides

5

3 6 3 6 *Serum non esterified fatty acids (NEFA)* Serum NEFA concentration was estimated by a method suggested by Faholt *et al* (1973)

Principle

Serum is extracted with chloroform heptane methanol mixture in the presence of a phosphate buffer to eliminate interference from phospholipids and the extract is shaken with a high density copper reagent at pH 8.1. The copper soaps remain in the upper organic layer from which an aliquot is removed and the copper content is determined colorimetrically with diphenyl carbazide.

Reagents required

- 1 Extraction solvent containing chloroform heptane methanol (5:5:1) was prepared
- 2 Phosphate buffer (pH 6.4 35 mmol/l) Two volumes of potassium dihydrogen phosphate (4.539 g/l) were mixed with one volume of disodium hydrogen phosphate d hydrate (5.958 g/l) to prepare the buffer
- 3 Stock copper solution (500mmol/l) 12.07 g of copper nitrate trihydrate ($\text{Cu NO}_3)_2 \cdot 3\text{H}_2\text{O}$) was dissolved in distilled water and the volume was made to 100 ml with distilled water
- 4 Triethanolamine solution (1mol/l) 10 ml of triethanolamine was diluted to 100ml with distilled water to prepare 1 mol/l solution
- 5 Sodium hydroxide solution (1 mol/l) 4 g of sodium hydroxide was dissolved in distilled water and the volume was made to 100 ml using distilled water
- 6 Copper reagent 10 ml of stock copper solution 10 ml of triethanolamine solution and 6 ml of sodium hydroxide solution were mixed and diluted to 100 ml with distilled water to which 33 g of sodium chloride was added and the pH was adjusted to 8.1 using 1 mol/l sodium hydroxide solution
- 7 1:5 Diphenylcarbazide solution (4 g/l in ethanol) 40 mg of Diphenylcarbazide was dissolved in 10 ml ethanol to which 0.1 ml of triethanolamine solution was added (prepared immediately before use)
- 8 Stock standard palmitic acid solution (2 mmol/l) 51.2 mg of palmitic acid was dissolved in the extraction solvent and the volume was made to 100 ml using extraction solvent This solution was stored in a tightly stoppered container
- 9 Working standard palmitic acid solution 5 ml of stock standard palmitic acid solution was diluted to 20 ml with extraction solvent to give a solution containing 500 $\mu\text{mol/l}$ (prepared freshly)

Procedure

- 1 To 50 μ l serum in a suitable stoppered centrifuge tube 1 ml phosphate buffer and 6 ml extraction solvent were added At the same time 50 μ l working standard palmitic acid solution was taken in another centrifuge tube to which 1 ml of phosphate buffer and 6 ml extraction solvent were added
- 2 The tubes were shaken vigorously for 90 seconds left undisturbed for 15 min and then centrifuged at 4000 rpm for 10 min
- 3 The buffer was carefully removed by suction and 5 ml of extraction solvent settled at the bottom of the tubes was transferred to a similar dry centrifuge tube to which 2 ml of copper reagent was added
- 4 The tubes were shaken vigorously for 5 min and then centrifuged at 3000 rpm for 5 min
- 5 Three ml of the upper layer was transferred to a tube containing 0.5 ml phenyl carbazide solution and mixed carefully
- 6 The reading was taken after 15 min at 550 nm in a spectrophotometer

3.6.4 Antioxidant profile

3.6.4.1 Blood catalase

Blood catalase activity was estimated by the method suggested by Aebi (1974)

Principle

In the ultraviolet range H_2O_2 shows a continual increase in absorption with decreasing wavelength The decomposition of H_2O_2 can be followed directly by the

decrease in extinction at 240 nm. The difference in extinction per unit time is a measure of the catalase activity.

Reagents used

1 Phosphate Buffer (50 mM pH 7)

Solution A Dissolved 1.7g of KH_2PO_4 in double distilled water (DDW) made up to 250 ml

Solution B Dissolved 4.45g of $\text{Na}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 500 ml DDW or 3.549g Na_2PO_4 in 500 ml DDW

Mixed 250 ml of solution A and 387.5 ml of solution B

Stored at 2°C

2 Hydrogen peroxide (30 mM)

H_2O_2 (0.34 ml) was dissolved in 100 ml phosphate buffer and this was prepared freshly

Procedure

Plasma was separated from heparin sed blood by centrifugation and sedimented RBC was washed three times with normal saline. A stock haemolysate containing 5 g % Hb was prepared by the addition of 4 parts of DDW to 200 μl of thick RBC sediment. Immediately before the assay 1:500 dilution of this concentrated haemolysate was prepared with phosphate buffer by adding 10 μl of haemolysate to 5 ml of phosphate buffer which was the working haemolysate. The Hb content of this working haemolysate was also determined by the method of Drabkin.

Wavelength 240nm (Ultraviolet range)

	Phosphate buffer	Haemolysate	H ₂ O ₂
Blank	1 ml	2 ml	
Test		2 ml	1 ml

Recorded the initial O D and O D at every 1 minute for 3 minutes. From the initial and final O D catalase activity was calculated using the formulae given below

$$k = \frac{2.303}{180} \times \log \left(\frac{\text{first reading}}{\text{last reading}} \right)$$

$$k / \text{ml} = k \times a$$

$$\text{Activity in } k / \text{g Hb} = \frac{k / \text{ml}}{b} \times \frac{1000}{180} = \frac{2.303 \times a}{b} \times \log \left(\frac{\text{first reading}}{\text{last reading}} \right)$$

a dilution factor $5 \times 500 = 2500$

b Hb content in blood (g / l)

3.6.4.2 Serum reduced glutathione (GSH)

Serum GSH level was determined by the method by Moron *et al* (1979)

Principle

GSH is measured by its reaction with 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) to give a yellow coloured complex with absorption maximum at 412 nm

Reagents used

1 Phosphate buffer (pH 8.0 2M)

Solution A Dissolved 3.12 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 100ml distilled water

Solution B Dissolved 28.39 g of Na_2HPO_4 in 100 ml distilled water

Mixed 5.3 ml of solution A and 94.7 ml of solution B

2 DTNB (0.6 mM)

Freshly dissolved 12 mg of DTNB in 50 ml of the buffer

3 Prepared 25 per cent and 5 per cent Trichloroacetic Acid (TCA) by dissolving 25 g TCA in 100 ml distilled water and 5 g TCA in 100 ml distilled water respectively

Procedure

A volume of 62.5 μl of 25% TCA was added to 0.25 ml of serum to precipitate proteins. The tubes were then cooled on ice for 5 minutes and 0.3 ml of 5% TCA was added. Centrifuged the tubes for 10 minutes at 1000 rpm. 0.15 ml of the supernatant was aspirated and the volume was made up to 0.5 ml using ng buffer and to that 1 ml DTNB was added. Absorbance of the solution at 412 nm was measured using spectrophotometer and reduced glutathione level was estimated using standard calibration curve. The standard curve of GSH was prepared by using concentrations varying from 10-60 nmol for each assay. The values were expressed as nmol / ml

3.6.4.3 Blood superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was estimated by the method of Winterbourn *et al* (1975)

Principle

The activity was measured based on the ability of superoxide dismutase to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide

Reagents used

- 1 Phosphate buffer (0.06M pH 7.8)

Solution A Dissolved 0.936 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 100 ml double distilled water (DDW)

Solution B Dissolved 0.95 g Na_2HPO_4 in 100 ml double distilled water (DDW)

Mixed 8.5 ml of solution A and 91.5 ml of solution B. This was done by slowly adding solution A to solution B checking pH periodically once pH of 7.8 was attained addition of solution A was stopped

- 2 EDTA NaCN solution

150 micro litre of NaCN was dissolved in 100 ml of 0.1 M EDTA

- 3 Nitro Blue Tetrazolium (NBT)

12.3 mg of NBT was dissolved in 10 ml of phosphate buffer

- 4 Riboflavin 2 mM solution

4.5 mg of riboflavin was dissolved in 100 ml of phosphate buffer

Procedure

A volume of 100 μ l of blood was taken and added to 900 μ l of cold distilled water at 4°C. To this 0.25 ml of chloroform and 0.5 ml of absolute alcohol were added and mixed thoroughly. Centrifuged at 18000 rpm for 60 minutes under refrigeration and the clear supernatant was used for SOD assay at 560 nm using spectrophotometer.

	Phosphate buffer	EDTA NaCN solution	NBT solution	Riboflavin	Supernatant
Control	2.650 ml	200 μ l	100 μ l	50 μ l	
Test	2.550 ml	200 μ l	100 μ l	50 μ l	100 μ l

Immediately after the addition of riboflavin the O.D. was taken at 560 nm in a spectrophotometer. Kept it for 15 minutes under illumination and at the end O.D. was taken again.

Calculation

$$\text{Percentage of inhibition} = \frac{\text{O.D. of control} - \text{O.D. of test}}{\text{O.D. of control}} \times 100$$

$$\text{Volume of supernatant having 50\% inhibition} = \frac{100}{\% \text{ inhibition}} \times 50$$

$$\text{Amount of haemoglobin (Hb) in A/B} = \frac{A}{1750} \times \text{Hb (in g per cent of blood)}$$

under running tap water. Added 4ml of N butanol and mixed well (rotor). Centrifuged at 1000 rpm for 5 minutes and separated the organic layer. Absorbance of supernatant at 532 nm against blank (n butanol) was taken using spectrophotometer and peroxidation level was found out using standard calibration curve constructed by using different concentrations of 1,1,3,3-tetra methoxy propane varying from 0.05 to 3 nmol/ml.

3.6.5 Serum electrolytes

3.6.5.1 Estimation of sodium and potassium Sodium and Potassium in the serum were determined by flame photometry (Mouldin *et al.* 1996).

3.6.5.2 Estimation of calcium magnesium and iron Calcium, Magnesium and Iron in the serum were estimated using Atomic Absorption Spectrophotometer (Perkin Elmer Model No 3110).

Elements	Wavelength (nm)	Slit (nm)	Flame gases	Sensitivity check(mg/l)
Ca	422.7	0.7	Air acetylene	4
Mg	285.2	0.7	Air acetylene	0.3
Fe	248.3	0.2	Air acetylene	5

3 6 6 Meat Cholesterol estimation

The lipid was extracted by the method suggested by Folch *et al* (1957)

- 1 Took whole meat sample
- 2 Weighed one g of meat sample
- 3 Minced the meat sample completely using stirrer
- 4 Added 5 ml of freshly prepared chloroform methanol (2 : 1) solution
- 5 Mixed and shook well
- 6 Added 5 ml of distilled water
- 7 Mixed and shook well
- 8 Centrifuged at 2500 rpm for 10 min
- 9 Removed the top layer (methanol) by suction
- 10 Removed the middle layer with cotton swab
- 11 The bottom layer chloroform contained cholesterol
- 12 Took 50 μ l of the bottom layer in a test tube and kept it in a hot water bath for evaporation of chloroform

The concentration of cholesterol was estimated by cholesterol phenol aminoantipyrine (CHOD PAP) method as suggested by Richmond (1973) using Ecoline® Kits (M/S E Merck India Limited Mumbai)

3 6 7 Proximate composition of meat

The proximate composition of white meat from cranial aspect of pectoral region was determined by the standard procedure prescribed by AOAC (1990) and the values were expressed in g per 100 g of meat. Three representative samples were taken from each group and the analyses were carried out in duplicate. Moisture in

fresh meat was determined by weight loss after 16 h drying in a conventional oven at 105°C

The fat content was determined in moisture free samples by an ether extraction procedure in an Automatic Solvent Extraction System (SOX plus Model SCS 6 Pelican Equipments Chennai India) Moisture free fat free samples were used to estimate the protein and ash content The protein content was determined by Block Digestion Method (KEL Plus Model KES 6L Pelican Equipments Chennai India) Ash was determined by weight loss after 2½ h drying in a muffle furnace at 600°C

All the chemicals used in this experiment were from Merck Co Mumbai and were of high quality analytical grade

3.7 STATISTICAL ANALYSIS

The data were analysed by using the statistical techniques Analysis of Variance and Paired t Test (Snedecor and Cochran 1994)

Results

4 RESULTS

The present study was undertaken to evaluate the physio-biochemical changes in broiler chicken fed a ration having complete replacement of unsalted dried fish with acid sludge (fish waste) and surimi waste powder

4.1 EFFECT ON BODY WEIGHT

The body weight (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given in table 3 and fig 1. The values in G I ranged from 420.00 g to 2500.00 g. The values in G II ranged from 270.00 g to 2300.00 g and in G III ranged from 330.00 g to 2500.00 g. There was a significant ($P < 0.01$) increase in body weight within the three treatment groups between 4th and 5th, 5th and 6th, 6th and 7th weeks of age. No significant ($P > 0.05$) difference was noted between G I, G II and G III at fourth, fifth, sixth and seventh weeks of age.

4.2 EFFECT ON FEED CONSUMPTION

The quantity of feed consumed (mean \pm S.E.) at weekly intervals for the three treatments are indicated in table 3 and fig 2. The values in G I ranged from 685.00 g to 1350.00 g. The values in G II ranged from 715.00 g to 1360.00 g and in G III ranged from 690.00 g to 1375.00 g. There was a significant ($P < 0.01$) increase in feed consumption within the three treatment groups between 4th and 5th, 5th and 6th, 6th and 7th weeks of age. No significant ($P > 0.05$) difference was noted between G I, G II and G III at fourth, fifth, sixth and seventh weeks of age.

Table 3 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on body weight feed consumption (from fourth to seventh week of age) and gible weight (at seventh week of age) of broiler chicken

Parameter	Body weight (g / bird) mean± S E (n 15)				Feed consumption (g / b rd) mean± S E (n 15)				Gible weight (g/bird) mean± S E (n 15)
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh	Seventh
G I	495 33 ^P ± 11 71	1118 60 ^P ± 67 59	1536 67 ^P ± 35 34	2033 33 ^P ± 61 08	783 00 ^P ± 3 18	1041 67 ^P ± 16 07	1218 67 ^P ± 20 33	1514 67 ^P ± 7 42	91 73 ^P ± 7 57
G II	477 33 ^P ± 17 44	1220 67 ^P ± 74 99	1503 33 ^P ± 48 91	1833 33 ^P ± 73 30	791 33 ^P ± 11 04	1075 33 ^P ± 15 99	1209 33 ^P ± 20 08	1316 67 ^P ± 8 79	87 87 ^P ± 7 02
G III	484 67 ^P ± 16 67	1146 67 ^P ± 48 65	1490 00 ^P ± 51 69	2043 33 ^P ± 63 27	790 00 ^P ± 13 24	1092 00 ^P ± 16 82	1239 33 ^P ± 13 23	1 08 00 ^P ± 9 41	87 33 ^P ± 6 28

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

1 P < 0.05 significant at 5% level

In columns means bearing same superscripts (p, q, r) do not differ significantly

2 P < 0.05 significant at 5% level

P < 0.01 significant at 1% level

In rows means within groups were compared between subsequent weeks

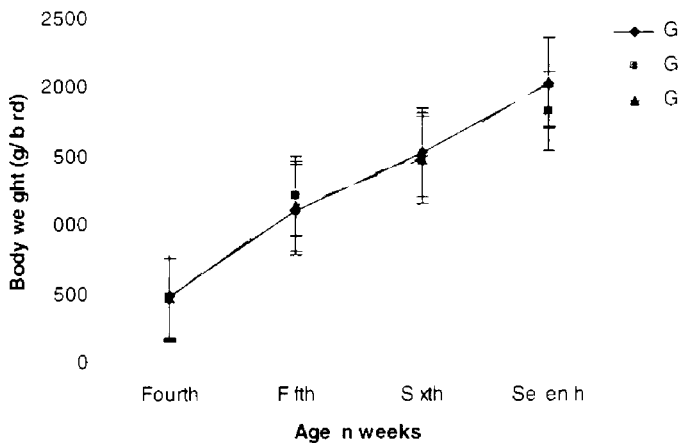


Fig 1 Effect of acid silage (fish waste) and surimi waste powder on body weight (g/bird) in broiler chicken

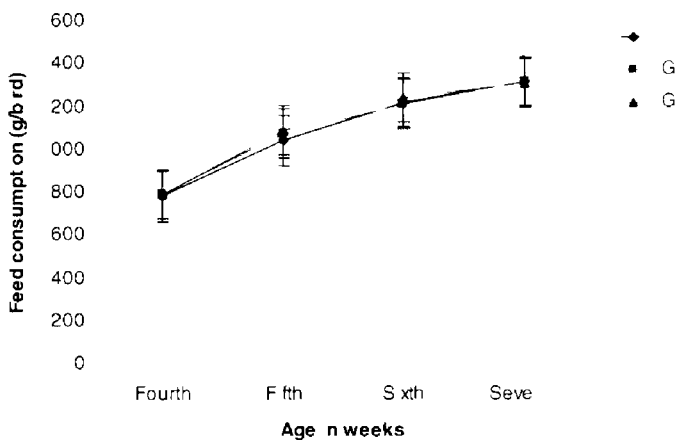


Fig 2 Effect of acid silage (fish waste) and surimi waste powder on feed consumption (g/bird) in broiler chicken
 G I control group (II acid silage (fish waste) fed group
 (III surimi waste powder fed group

4.3 EFFECT ON GIBLET WEIGHT

The mean values of gilet weight for the three treatments are on table 3. The values ranged from 45.00 to 130.00 g in G I, 35.00 to 124.00 g in G II and 40.00 to 120.00 g in G III. No significant ($P > 0.05$) difference was observed between the three groups during the study.

4.4 EFFECT OF ACID SILAGE AND SURIMI WASTE POWDER ON HAEMATOLOGICAL PARAMETERS

4.4.1 Effect on haemoglobin (Hb) concentration

The Hb count (mean \pm S.E.) for the three groups for 4th, 5th, 6^h and 7th weeks are given on table 4 and fig 3. The values in G I ranged from 5.00 to 9.50 g %. The values in G II varied from 5.00 to 10.00 g % and in G III ranged from 5.00 to 10.00 g %. At 4^h, 5th, 6^h and 7^h weeks of age the Hb concentration did not vary significantly ($P > 0.05$) among the three groups. There was a significant ($P < 0.01$) increase between 4^h and 5^h, 5th and 6^h and 6th and 7^h weeks of age in the control and experimental groups.

4.4.2 Effect on Volume of Packed Red Cells (VPRC)

The VPRC count (mean \pm S.E.) for the three groups for 4^h, 5th, 6^h and 7^h weeks of age are given on table 4 and fig 4. The values in G I ranged from 21.60 to 38.80 %. The values in G II ranged from 20.34 to 37.80 % and in G III ranged from 23.64 to 37.50 %. There was a significant ($P < 0.01$) increase in VPRC within group II between 4^h and 5th weeks and also 6th and 7th weeks of age. VPRC was

Table 4 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on haemoglobin (Hb) and volume of packed red cells (VPRC) of broiler chicken from fourth to seventh week of age

Parameter	Hb (g %)				VPRC (%)			
	mean± S E (n = 15)				mean± S E (n = 15)			
Age (wks)	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G I	5.67 ^P ± 0.09	6.63 ^P ± 0.14	7.43 ^P ± 0.15	8.53 ^P ± 0.2	28.90 ^P ± 1.18	29.16 ^P ± 0.99	31.37 ^P ± 0.72	34.07 ^P ± 0.67
G II	5.60 ^P ± 0.08	6.40 ^P ± 0.11	7.20 ^P ± 0.14	8.57 ^P ± 0.18	27.8 ^P ± 0.88	29.95 ^P ± 1.03	31.69 ^P ± 0.64	33.59 ^P ± 0.62
G III	5.50 ^P ± 0.09	6.60 ^P ± 0.15	7.63 ^P ± 0.17	8.73 ^P ± 0.24	30.35 ^P ± 0.66	30.97 ^P ± 0.96	32.02 ^P ± 0.89	33.49 ^P ± 0.71

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

- 1 P < 0.05 significant at 5% level
In columns means bearing same superscripts (p, q, r) do not differ significantly
- 2 P < 0.05 significant at 5% level
P < 0.01 significant at 1% level
In rows means within groups were compared between subsequent weeks

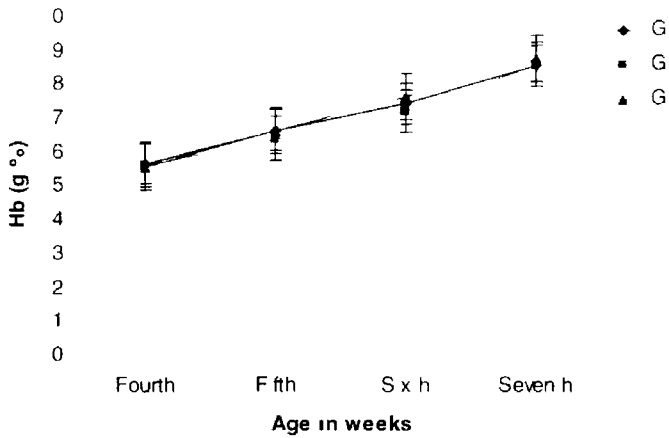


Fig. 3 Effect of acid silage (fish waste) and surimi waste powder on Hb (g %) level in broiler chicken

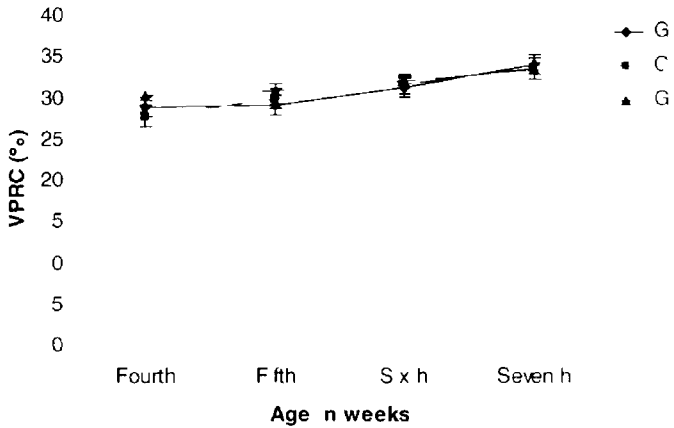


Fig. 4 Effect of acid silage (fish waste) and surimi waste powder on VPRC (%) level in broiler chicken
 (I control group (II acid silage fish waste fed group
 (III surimi waste powder fed group

significantly ($P < 0.05$) increased within group I between 5th and 6th weeks and there was a significant ($P < 0.01$) increase between 6th and 7th weeks of age. There was a significant ($P < 0.01$) increase in VPRC within group III between 5th and 6th weeks and also 6th and 7th weeks of age. There was no significant ($P > 0.05$) difference in VPRC between G I, G II and G III at fourth, fifth, sixth and seventh weeks of age.

4.4.3 Effect on total erythrocyte count (TEC)

The TEC (mean \pm S.E.) for the three groups for 4th, 5th, 6th and 7th weeks of age are given on table 5 and fig 5. The values in G I ranged from 0.92 to 3.25 ($\times 10^6 / \mu\text{l}$). The values in G II ranged from 1.13 to 3.24 ($\times 10^6 / \mu\text{l}$) and in G III ranged from 0.64 to 5.18 ($\times 10^6 / \mu\text{l}$). There was no significant ($P > 0.05$) difference in the values of TEC between G I, G II and G III at fourth, fifth, sixth and seventh weeks of age. There was a significant ($P < 0.01$) increase in the TEC within the three groups between 4th and 5th, 5th and 6th, 6th and 7th weeks of age.

4.4.4 Effect on total leucocyte count (TLC)

The TLC (mean \pm S.E.) for the three groups for 4th, 5th, 6th and 7th weeks of age are given on table 5 and fig 6. The values in G I ranged from 8.00 to 29.00 ($\times 10^3 / \mu\text{l}$). The values in G II ranged from 9.00 to 29.00 ($\times 10^3 / \mu\text{l}$) and in G III ranged from 9.00 to 30.00 ($\times 10^3 / \mu\text{l}$). There was a significant ($P < 0.01$) increase in TLC within the groups between 4th and 5th, 5th and 6th, 6th and 7th weeks of age. There was no significant ($P > 0.05$) difference in TLC between G I, G II and G III at fourth, fifth, sixth and seventh weeks of age.

Table 5 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on total erythrocyte count (TEC) and total leucocyte count (TLC) of broiler chicken from fourth to seventh week of age

Parameter	TEC ($\times 10^6/\mu\text{l}$) mean \pm S E (n 15)				TLC ($\times 10^3/\mu\text{l}$) mean \pm S E (n 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
Age (wks)								
Groups								
G I	1.48 ^P \pm 0.06	1.78 ^P \pm 0.09	2.13 ^P \pm 0.06	2.83 ^P \pm 0.07	15.20 ^P \pm 0.99	17.80 ^P \pm 1.01	20.27 ^P \pm 1.12	22.00 ^P \pm 1.04
G II	1.33 ^P \pm 0.05	1.83 ^P \pm 0.05	2.18 ^P \pm 0.04	2.79 ^P \pm 0.06	16.53 ^P \pm 1.37	19.53 ^P \pm 1.21	21.80 ^P \pm 1.07	24.60 ^P \pm 0.97
G III	1.39 ^P \pm 0.07	1.71 ^P \pm 0.12	2.09 ^P \pm 0.07	2.85 ^P \pm 0.06	15.27 ^P \pm 1.02	18.00 ^P \pm 0.20	20.60 ^P \pm 0.95	23.40 ^P \pm 0.81

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

1 P < 0.05 significant at 5% level

In columns means bearing same superscripts (p q r) do not differ significantly

2 P < 0.05 significant at 5% level

P < 0.01 significant at 1% level

In rows means within groups were compared between subsequent weeks

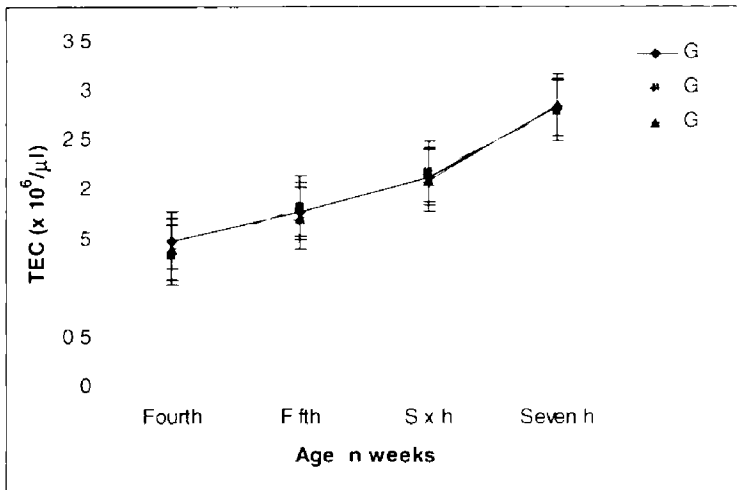


Fig. 5 Effect of acid silage (fish waste) and surimi waste powder on TEC ($\times 10^6 / \mu\text{l}$) in broiler chicken

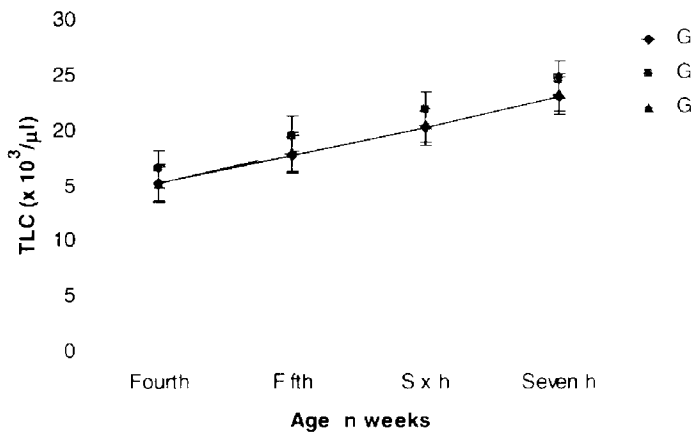


Fig. 6 Effect of acid silage (fish waste) and surimi waste powder on TLC ($\times 10^3 / \mu\text{l}$) in broiler chicken
 C I control group C II acid silage (fish waste) fed group
 G III surimi waste powder fed group

4.5 EFFECT OF ACID SILAGE (FISH WASTE) AND SURIMI WASTE ON BIOCHEMICAL PARAMETERS

4.5.1 Effect on serum protein profile

4.5.2.1 Effect on serum total protein

The total protein concentration (mean \pm S.E.) for the three groups for 4th, 5th, 6th and 7th weeks of age are given on table 6 and fig 7. The values in G I ranged from 0.80 to 3.70 g/dl. The values in G II ranged from 0.90 to 3.60 g/dl and in G III ranged from 0.90 to 3.70 g/dl. There was no significant ($P > 0.05$) difference between the G I, G II and G III in the serum albumin level at fourth, fifth, sixth and seventh weeks of age. Within the groups there was a significant ($P < 0.01$) increase between 4th and 5th, 6th and 7th weeks of age. There was a significant ($P < 0.01$) increase between 4th and 5th, 6th and 7th weeks of age in all the three groups. Between 5th and 6th weeks of age there was a significant ($P < 0.01$) increase in G II and G III but in the case of G I a significant ($P < 0.05$) increase was noted during the period.

4.5.2.2 Effect on serum albumin

The albumin concentration (mean \pm S.E.) for the three groups for 4th, 5th, 6th and 7th weeks of age are given on table 6 and fig 8. The values in G I ranged from 0.30 to 1.90 g/dl. The values in G II ranged from 0.50 to 1.90 g/dl and in G III ranged from 0.40 to 1.90 g/dl. There was no significant ($P > 0.05$) difference between the G I, G II and G III in the serum albumin level at fourth, fifth, sixth and seventh weeks of age. Within the groups there was a significant ($P < 0.01$) increase between

Table 6 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum total protein and albumin of broiler chicken from fourth to seventh week of age

Parameter	Total protein (g/dl) mean± S E (n 15)				Albumin (g/dl) mean± S E (n 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
Age (wks)								
Groups								
G I	1.95 ^P ± 0.12	2.20 ^P ± 0.06	2.54 ^P ± 0.12	3.05 ^P ± 0.12	2.4 ^P ± 0.04	1.18 ^P ± 0.07	1.31 ^P ± 0.06	5.9 ^P ± 0.05
G II	1.69 ^P ± 0.11	2.05 ^P ± 0.11	2.34 ^P ± 0.13	2.86 ^P ± 0.11	1.16 ^P ± 0.04	1.07 ^P ± 0.06	1.17 ^P ± 0.07	1.49 ^P ± 0.07
G III	1.67 ^P ± 0.09	2.01 ^P ± 0.09	2.46 ^P ± 0.11	3.01 ^P ± 0.11	1.9 ^P ± 0.04	1.02 ^P ± 0.04	2.8 ^P ± 0.05 ^P	6.1 ^P ± 0.05

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

1 P < 0.05 significant at 5% level

In columns means bearing same superscripts (p, q) do not differ significantly

2 P < 0.05 significant at 5% level

P < 0.01 significant at 1% level

In rows means within groups were compared between subsequent weeks

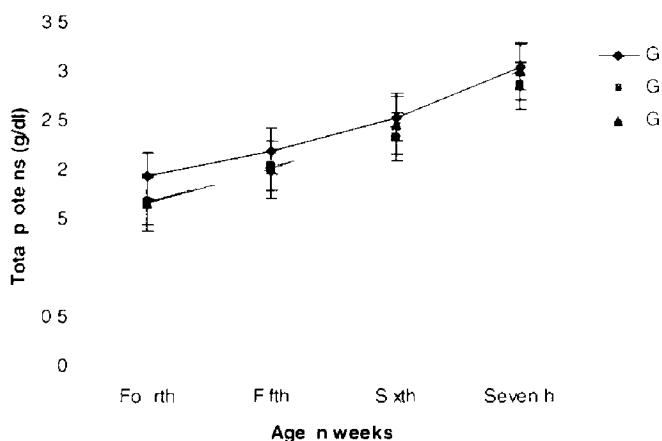


Fig 7 Effect of acid silage (fish waste) and surimi waste powder on serum total protein level (g/dl) in broiler chicken

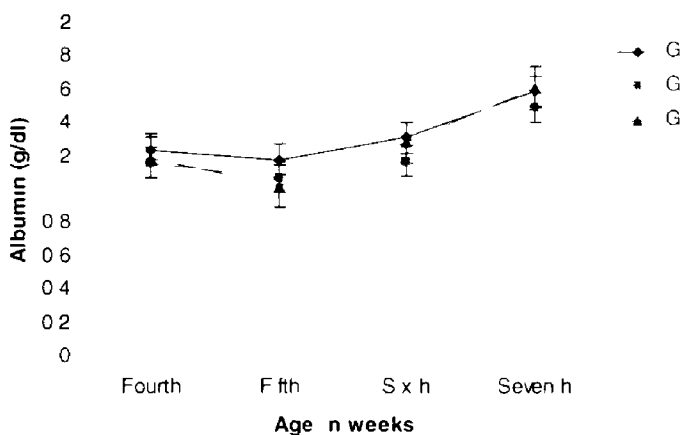


Fig 8 Effect of acid silage (fish waste) and surimi waste powder on serum albumin level (g/dl) in broiler chicken
 G I - control group
 G II - acid silage (fish waste) fed group
 G III - surimi waste powder fed group

4th and 5th 6th and 7th weeks of age In the case of G I there was no significant ($P > 0.05$) difference between 5th and 6th weeks of age but there was a significant ($P < 0.01$) increase in the case of G II and G III

4.5.1.2 Effect on serum globulin

The globulin concentration (mean \pm S.E.) for the three groups for 4th 5th 6th and 7th weeks of age are given on table 7 and fig 9 The values in G I varied from 0.50 to 1.80 g/dl The values in G II varied from 0.40 to 1.70 g/dl and in G III varied from 0.50 to 1.80 g/dl There was a significant difference between groups in the globulin value during the 4th week of age Here G I was significantly ($P < 0.05$) different from G II and G III There was no significant ($P > 0.05$) difference between the groups during the other weeks of age There was a significant ($P < 0.01$) increase within the G II and G III between 4th and 5th 5th and 6th 6th and 7th weeks of age There was a significant ($P < 0.05$) increase between 5th and 6th weeks in birds of G I In G I there was also a significant ($P < 0.01$) increase in the globulin value between 6th and 7th weeks of age

4.5.1.3 Effect on albumin globulin ratio

The albumin globulin ratio (mean \pm S.E.) at 4th 5th 6th and 7th weeks of age for the three treatment groups are given on table 7 and fig 10 The values in G I ranged from 0.27 to 1.29 The values in G II ranged from 0.54 to 1.27 and in G III ranged from 0.60 to 1.45 There was a significant ($P < 0.05$) increase between 4th and 5th weeks and also 6th and 7th weeks of age in the birds of group I There was no significant ($P > 0.05$) difference between the three groups at fourth fifth sixth and seventh weeks of age



Table 7 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum globulin and albumin globulin ratio of broiler chicken from fourth to seventh week of age

Parameter	Globulin (g/dl) mean ± S E (n = 15)				A G mean ± S E (n = 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
Age (wks)								
Groups								
G I	1.07 ^P ± 0.08	1.02 ^P ± 0.01	1.23 ^P ± 0.06	1.45 ^P ± 0.06	0.91 ^P ± 0.09	1.14 ^P ± 0.06	1.07 ^P ± 0.02	1.11 ^P ± 0.02
G II	0.80 ^q ± 0.06	0.99 ^P ± 0.06	1.17 ^P ± 0.06	1.37 ^P ± 0.05	1.11 ^P ± 0.08	1.04 ^P ± 0.07	1.02 ^P ± 0.05	1.11 ^P ± 0.05
G III	0.83 ^q ± 0.06	0.99 ^P ± 0.06	1.18 ^P ± 0.06	1.39 ^P ± 0.07	1.05 ^P ± 0.06	1.07 ^P ± 0.05	1.11 ^P ± 0.04	1.19 ^P ± 0.06

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

1. P < 0.05 significant at 5% level
In columns means bearing same superscripts (p, q) do not differ significantly
2. P < 0.05 significant at 5% level
P < 0.01 significant at 1% level
In rows means within groups were compared between subsequent weeks

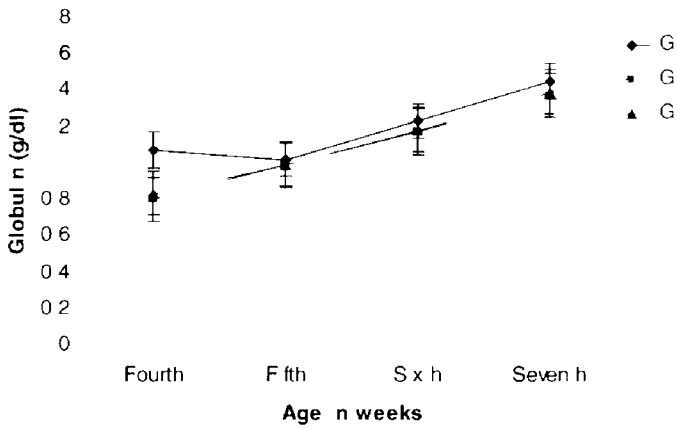


Fig. 9 Effect of acid silage (fish waste) and surimi waste powder on serum globulin level (g/dl) in broiler chicken

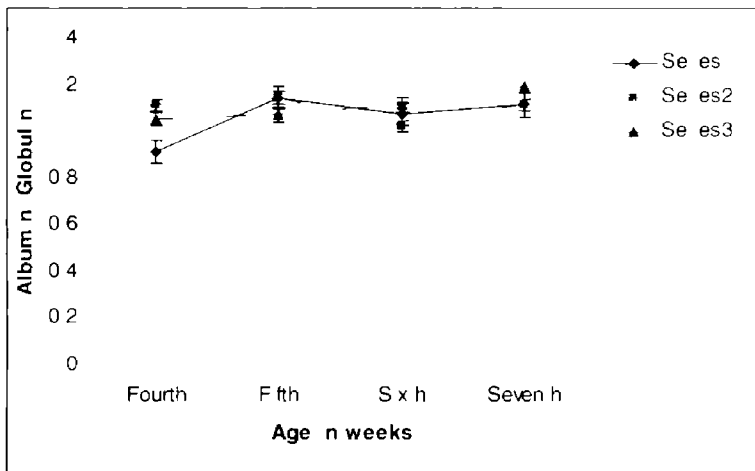


Fig. 10 Effect of acid silage (fish waste) and surimi waste powder on serum albumin globulin ratio in broiler chicken
 (I control group (II acid silage fish waste fed group
 (III surimi waste powder fed group

4.5.2 Effect on serum enzymes

4.5.2.1 Effect on alanine amino transferase (ALT)

The ALT values (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 8 and fig 11. The values in G I ranged from 4.00 to 24.00 U/l. The values in G II ranged from 3.00 to 26.00 U/l and in G III ranged from 4.00 to 22.00 U/l. There was no significant ($P > 0.05$) difference between the three groups of birds at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) difference was noted within G I, G II and G III between 4th and 5th, 6th and 7th weeks of age. There was a significant difference ($P < 0.05$) within G I and G III between 5th and 6th weeks of age.

4.5.2.2 Effect on aspartate amino transferase (AST)

The values of AST (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 8 and fig 12. The values in G I ranged from 99.00 to 255.00 U/l. The values in G II ranged from 71.00 to 246.00 U/l and in G III ranged from 98.00 to 290.00 U/l. There was a significant ($P < 0.01$) increase within G I between 5th and 6th weeks of age and in G II between 4th and 5th weeks of age. A significant ($P < 0.05$) increase was observed within G II between 5th and 6th weeks of age and within G III between 4th and 5th weeks of age. No significant ($P > 0.05$) difference was observed between the G I, G II and G III at fourth, fifth, sixth and seventh weeks of age.

Table 8 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum alanine amino transferase (ALT) and aspartate amino transferase (AST) of broiler chicken from fourth to seventh week of age

Parameter	ALT (U/l) mean± S E (n 15)				AST (U/l) mean± S E (n 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G I	10.73 ^P ± 0.71	13.20 ^P ± 0.45	5.07 ^P ± 0.78	19.07 ^P ± 0.73	139.53 ^P ± 6.03	154.87 ^P ± 5.93	175.07 ^P ± 4.41	82.47 ^P ± 8.64
G II	11.07 ^P ± 1.11	14.53 ^P ± 0.75	15.27 ^P ± 1.29	19.47 ^P ± 0.90	136.47 ^P ± 6.93	154.47 ^P ± 6.93	173.3 ^P ± 8.29	180.60 ^P ± 6.38
G III	10.27 ^P ± 0.74	13.13 ^P ± 0.68	15.33 ^P ± 0.71	19.20 ^P ± 0.57	130.60 ^P ± 4.87	155.07 ^P ± 7.99	160.07 ^P ± 6.32	175.93 ^P ± 8.73

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

P < 0.05 significant at 5% level

In columns means bearing same superscript (p, q) do not differ significantly

2 P < 0.05 significant at 5% level

P < 0.01 significant at 1% level

In rows means within groups were compared between subsequent weeks

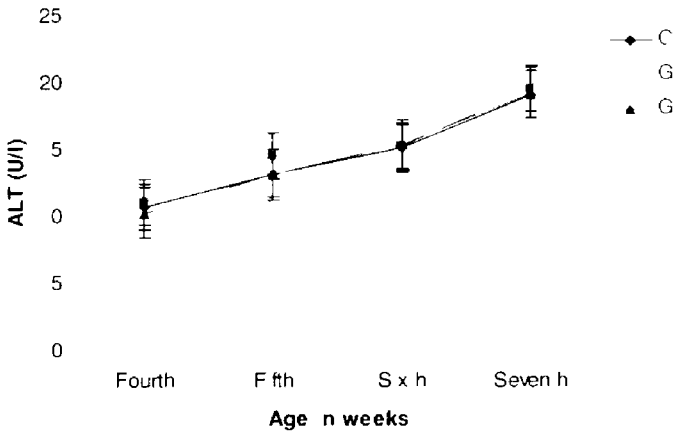


Fig. 11 Effect of acid silage (fish waste) and surimi waste powder on serum ALT level (U/l) in broiler chicken

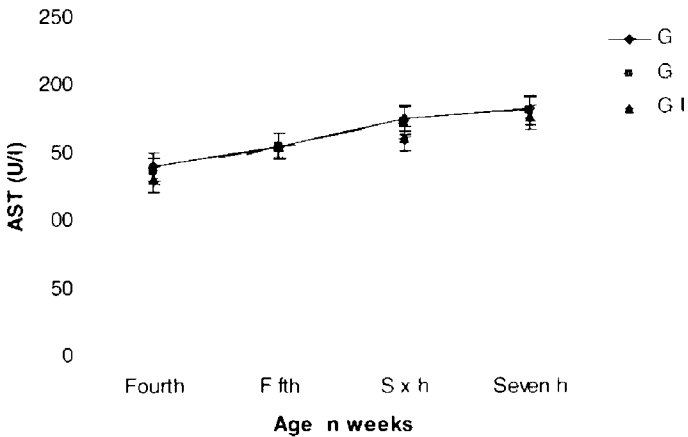


Fig. 12 Effect of acid silage (fish waste) and surimi waste powder on serum AST level (U/l) in broiler chicken
 (I) control group (II) acid silage (fish waste) fed group
 (III) surimi waste powder fed group

4.5.3 Effect on serum lipid profile

4.5.3.1 Effect on total lipids

The values of the effect of acid silage and surimi waste powder on serum total lipids (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatment groups are given on table 9 and fig 13. The values in G I ranged from 251.00 to 858.00 mg/dl. The values in G II ranged from 182.00 to 914.00 mg/dl and in G III ranged from 196.00 to 993.00 mg/dl. Between the three groups there was no significant ($P > 0.05$) difference in the values of total lipids at fourth, fifth, sixth and seventh weeks of age. Within G I and G II there was a significant ($P < 0.01$) increase between 4th and 5th, 5th and 6th weeks of age. In the case of group I birds a significant ($P < 0.05$) increase was observed between 6th and 7th weeks. In G II there was a significant ($P < 0.05$) increase between 4th and 5th weeks of age and a significant ($P < 0.01$) increase between 5th and 6th, 6th and 7th weeks of age.

4.5.3.2 Effect on triglycerides

The values of triglycerides (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 9 and fig 14. The values in G I ranged from 41.00 to 104.00 mg/dl. The values in G II ranged from 40.00 to 100.00 mg/dl and in G III ranged from 42.00 to 102.00 mg/dl. There was no significant ($P > 0.05$) difference observed between the values of the three groups at fourth, fifth, sixth and seventh weeks of age. Within the groups in G I and G II a significant ($P < 0.01$) increase was found between 4th and 5th, 6th and 7th weeks of age. In the G III there was a significant ($P < 0.01$) increase between 5th and 6th, 6th and 7th weeks of age.

Table 9 Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum total lipids and triglycerides of broiler chicken from fourth to seventh week of age

Parameter	Total lipids (mg/dl) mean± S E (n = 15)				Triglycerides (mg/dl) mean± S E (n = 15)			
	Age (wks) Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G I	375.80 ^P ± 25.84	491.33 ^P ± 31.61	622.07 ^P ± 28.54	725.13 ^P ± 24.99	60.13 ^P ± 3.40	68.60 ^P ± 2.75	75.00 ^P ± 2.71	85.07 ^P ± 3.27
G II	407.13 ^P ± 38.55	509.73 ^P ± 28.27	622.60 ^P ± 39.23	710.87 ^P ± 38.65	64.20 ^P ± 3.30	69.00 ^P ± 3.29	73.00 ^P ± 3.09	88.07 ^P ± 4.49
G III	416.13 ^P ± 38.79	525.87 ^P ± 43.38	633.60 ^P ± 49.88	733.53 ^P ± 31.66	56.13 ^P ± 4.02	60.27 ^P ± 2.88	66.73 ^P ± 3.06	77.13 ^P ± 2.50

G I Control group G II Acid silage (fish waste) fed group G III Surimi waste powder fed group

P < 0.05 significant at 5% level

In columns means bearing same superscripts (p, q, r) do not differ significantly

2 P < 0.05 significant at 5% level

P < 0.01 significant at 1% level

In rows means within groups were compared between subsequent weeks

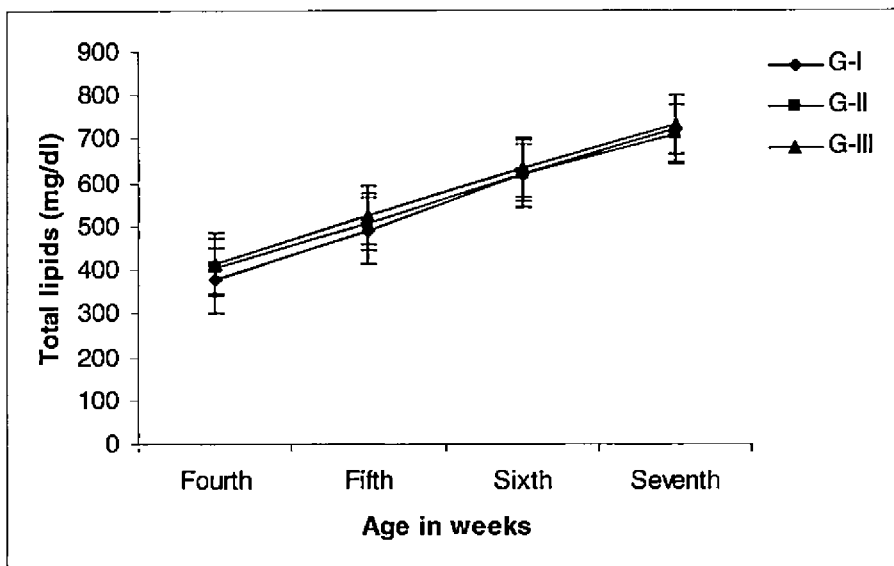


Fig. 13. Effect of acid silage (fish waste) and surimi waste powder on serum total lipid level (mg/dl) in broiler chicken

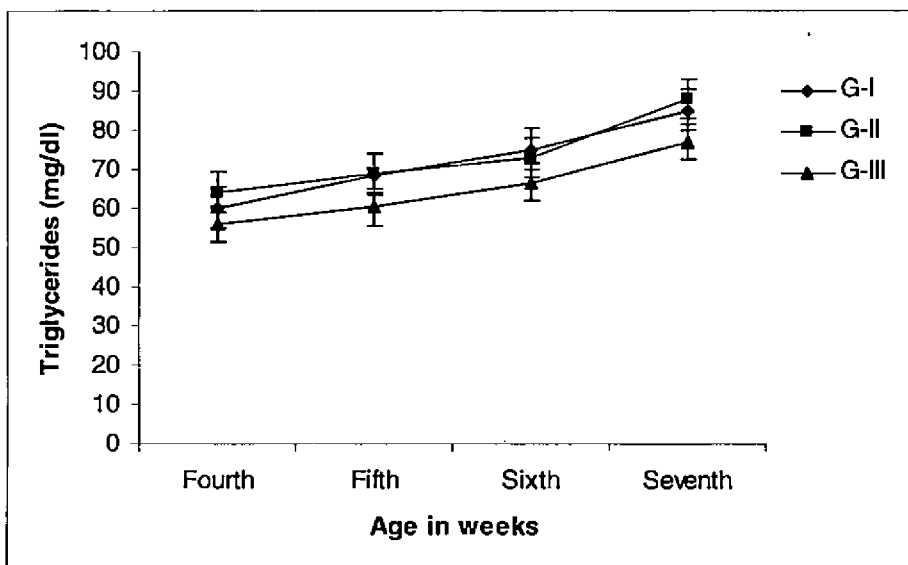


Fig. 14. Effect of acid silage (fish waste) and surimi waste powder on serum triglyceride level (mg/dl) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

4.5.3.3. *Effect on high density lipoproteins (HDL)*

The HDL values (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 10 and fig.15. The values in G-I ranged from 20.00 to 74.00 mg/dl. The values in G-II ranged from 22.00 to 74.00 mg/dl and in G-III ranged from 23.00 to 70.00 mg/dl. There was a significant ($P > 0.05$) difference between the groups during 7th week of age. G-I was significantly ($P < 0.05$) different from G-II. A significant ($P < 0.01$) increase was observed within the groups between 4th and 5th, 5th and 6th, 6th and 7th weeks of age.

4.5.3.4. *Effect on very low density lipoproteins (VLDL)*

The values of VLDL (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 10 and fig.16. The values in G-I ranged from 8.20 to 20.80 mg/dl. The values in G-II ranged from 8.00 to 27.80 mg/dl and in G-III ranged from 7.20 to 20.40 mg/dl. There was no significant ($P > 0.05$) difference between the three groups in the values of VLDL at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was observed within G-I and G-II between 4th and 5th, 6th and 7th weeks of age. There was a significant ($P < 0.01$) increase within G-III between 5th and 6th, 6th and 7th weeks of age.

4.5.3.5. *Effect on non-esterified fatty acids (NEFA)*

The values of NEFA (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 11 and fig.17. The values in G-I ranged from 148.92 to 697.28 $\mu\text{mol/l}$. The values in G-II ranged from 129.40 to 698.65 $\mu\text{mol/l}$.

Table 10. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum high density lipoproteins (HDL) and very low density lipoproteins (VLDL) of broiler chicken from fourth to seventh week of age.

Parameter	HDL (mg/dl) mean± S.E. (n = 15)				VLDL (mg/dl) mean± S.E. (n = 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
Age (wks)								
Groups								
G-I	32.60 ^P ± 2.28	40.80 ^{P..} ± 1.65	47.13 ^{P..} ± 3.00	62.07 ^{P..} ± 2.02	12.03 ^P ± 0.68	13.72 ^{P..} ± 0.55	15.01 ^P ± 0.54	16.95 ^{P..} ± 0.67
G-II	32.47 ^P ± 2.09	39.33 ^{P..} ± 2.84	47.27 ^{P..} ± 2.85	54.47 ^{Pq..} ± 2.30	12.85 ^P ± 0.67	13.80 ^{P..} ± 0.66	14.60 ^P ± 0.62	17.61 ^{P..} ± 0.90
G-III	31.00 ^P ± 2.28	38.87 ^{P..} ± 2.76	46.47 ^{P..} ± 2.87	58.00 ^{Pq..} ± 1.84	11.23 ^P ± 0.80	12.05 ^P ± 0.58	13.35 ^{P..} ± 0.61	15.43 ^{P..} ± 0.50

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. P < 0.05, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. .P < 0.05, significant at 5 % level.
..P < 0.01, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

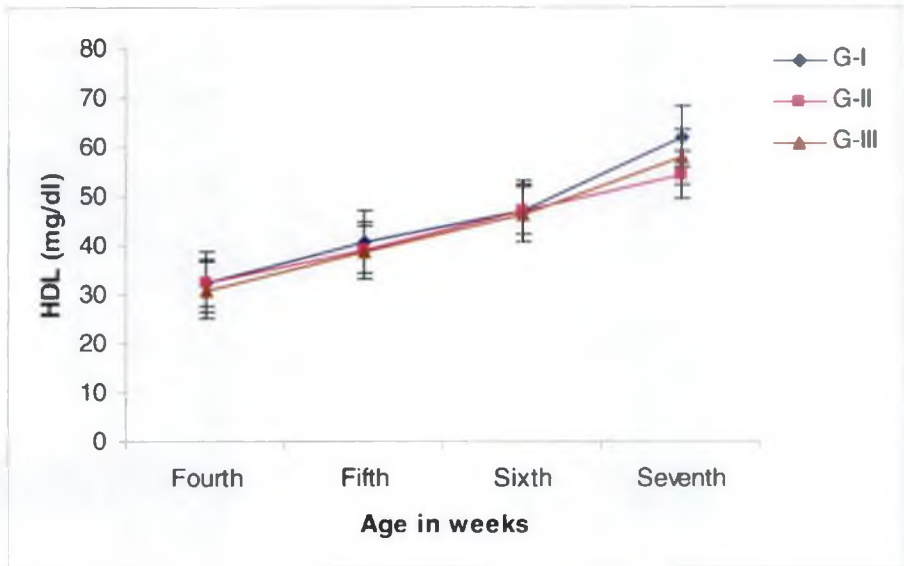


Fig. 15. Effect of acid silage (fish waste) and surimi waste powder on serum HDL level (mg/dl) in broiler chicken

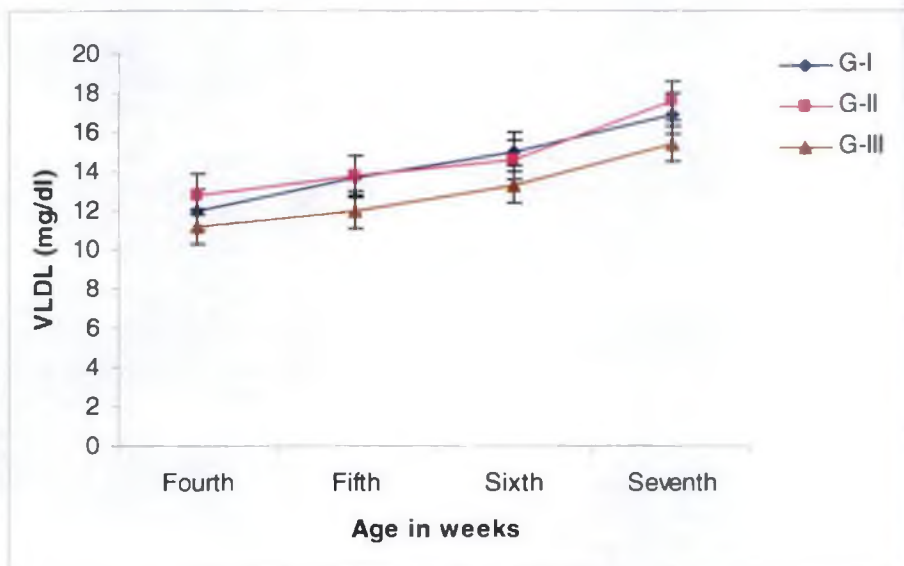


Fig. 16. Effect of acid silage (fish waste) and surimi waste powder on serum VLDL level (mg/dl) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

Table 11. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum non esterified fatty acids (NEFA) and total cholesterol of broiler chicken from fourth to seventh week of age.

Parameter	NEFA ($\mu\text{mol/l}$) mean \pm S.E. (n = 15)				Total cholesterol (mg/dl) mean \pm S.E. (n = 15)				
	Age (wks)	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G-I		310.16 ^P \pm 23.03	416.42 ^{P..} \pm 26.61	470.64 ^{P.} \pm 31.90	590.06 ^{P..} \pm 20.00	70.93 ^P \pm 5.22	76.33 ^{P.} \pm 4.62	85.47 ^{P..} \pm 4.13	96.27 ^{P..} \pm 5.30
G-II		297.24 ^P \pm 20.62	354.46 ^{P..} \pm 23.15	437.07 ^{P..} \pm 27.39	547.81 ^{P..} \pm 26.72	73.53 ^P \pm 3.84	82.73 ^{P..} \pm 4.21	92.80 ^{P..} \pm 4.52	103.73 ^{P..} \pm 6.92
G-III		307.70 ^P \pm 24.53	373.21 ^{P..} \pm 29.43	452.70 ^{P..} \pm 27.39	553.71 ^{P..} \pm 27.16	67.87 ^P \pm 4.30	78.53 ^{P..} \pm 5.12	87.13 ^{P..} \pm 5.21	99.00 ^{P..} \pm 5.21

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. $P < 0.05$, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. [.] $P < 0.05$, significant at 5 % level.
^{..} $P < 0.01$, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

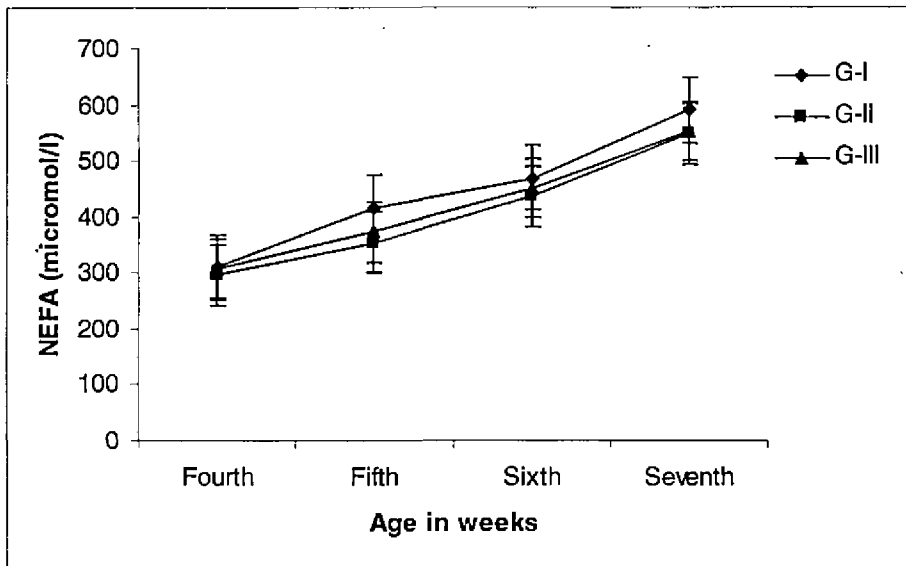


Fig. 17. Effect of acid silage (fish waste) and surimi waste powder on serum NEFA level (micromol/l) in broiler chicken

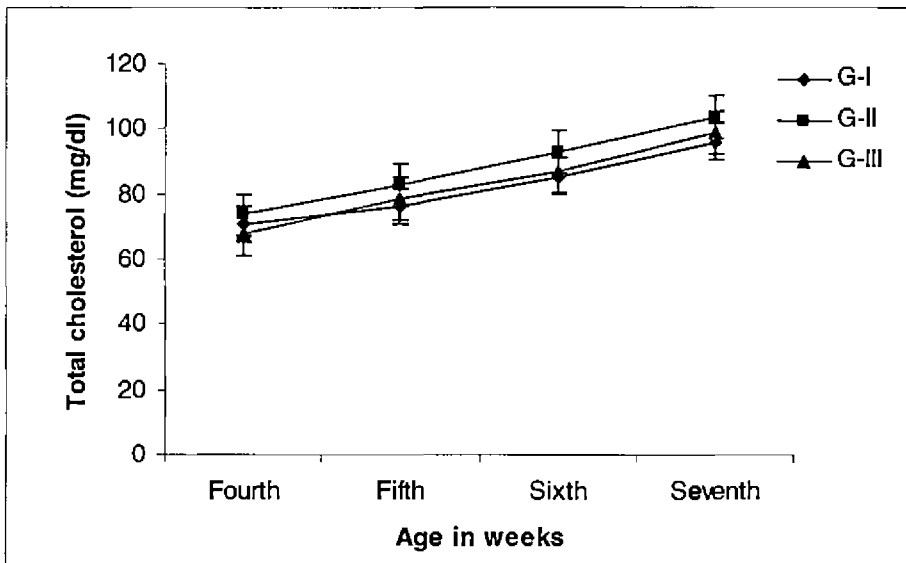


Fig. 18. Effect of acid silage (fish waste) and surimi waste powder on serum total cholesterol level (mg/dl) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

and in G-III ranged from 156.38 to 775.00 $\mu\text{mol/l}$. There was no significant ($P > 0.05$) difference between the three groups at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was noted within the G-II and G-III between 4th and 5th, 5th and 6th, 6th and 7th weeks of age. In G-I, there was a significant ($P < 0.05$) increase between 5th and 6th weeks of age while a significant ($P < 0.01$) increase was observed between 4th and 5th, 6th and 7th weeks of age.

4.5.3.6. *Effect on total cholesterol*

The total cholesterol values (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 11 and fig.18. The values in G-I ranged from 38.00 to 150.00 mg/dl. The values in G-II ranged from 51.00 to 143.00 mg/dl and in G-III ranged from 43.00 to 149.00 mg/dl. There was a significant ($P < 0.01$) increase within G-I, G-II and G-III between 4th and 5th, 5th and 6th, 6th and 7th weeks of age. There was no significant ($P > 0.05$) difference between G-I, G-II and G-III at fourth, fifth, sixth and seventh weeks of age.

4.5.4. **Effect on antioxidant status:**

4.5.4.1. *Effect on superoxide dismutase (SOD)*

The values of SOD (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 12 and fig.19. The values in G-I ranged from 188.00 to 367.00 U/g Hb. The values in G-II ranged from 179.00 to 330.00 U/g Hb and in G-III ranged from 185.00 to 329.00 U/g Hb. A significant ($P < 0.01$) increase was observed within group II between 5th and 6th weeks, 6th and 7th weeks and between 4th and 5th weeks, there was a significant ($P < 0.05$) increase noted. A significant ($P < 0.01$) increase was noted within G-I and G-III between 4th and 5th

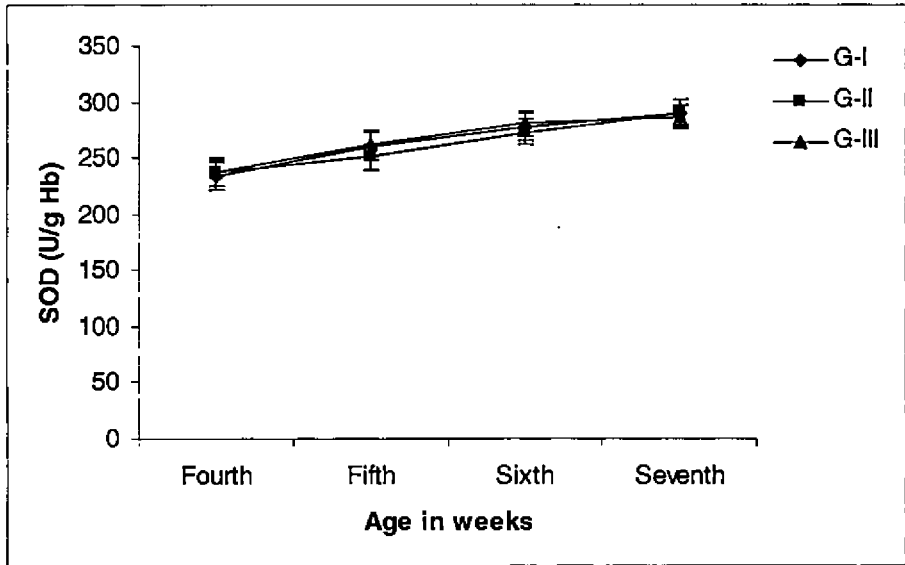


Fig. 19. Effect of acid silage (fish waste) and surimi waste powder on blood SOD level (U/g Hb) in broiler chicken

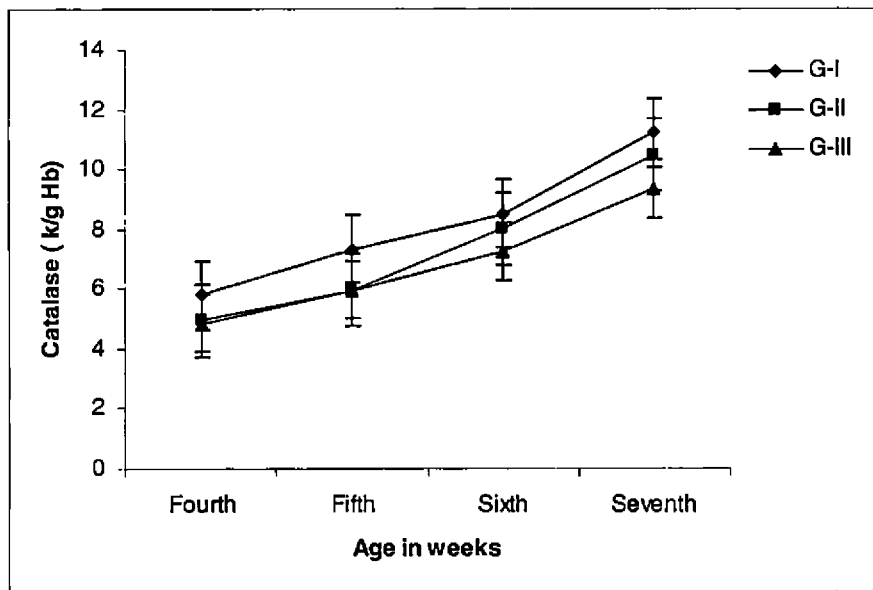


Fig. 20. Effect of acid silage (fish waste) and surimi waste powder on blood catalase level (k/g Hb) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

Table 12. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on superoxide dismutase (SOD) and catalase of broiler chicken from fourth to seventh week of age.

Parameter	SOD (U/g Hb) mean± S.E. (n = 15)				Catalase (k/g Hb) mean± S.E. (n = 15)			
	Age (wks) Group	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth
G-I	234.47 ^P ± 8.24	260.33 ^{P..} ± 8.90	278.93 ^{P.} ± 9.76	290.80 ^{P.} ± 7.44	11.60 ^P ± 0.77	14.68 ^{P.} ± 0.95	17.04 ^P ± 1.11	22.44 ^{P..} ± 1.38
G-II	238.07 ^P ± 9.14	251.27 ^{P.} ± 8.06	273.73 ^{P..} ± 8.62	291.20 ^{P..} ± 6.97	9.88 ^P ± 0.44	11.96 ^P ± 0.45	16.06 ^{P.} ± 0.76	20.96 ^{P..} ± 1.12
G-III	237.13 ^P ± 7.81	263.00 ^{P..} ± 8.11	281.13 ^{P.} ± 8.33	287.00 ^P ± 5.83	9.74 ^P ± 0.41	11.94 ^{P..} ± 0.38	14.52 ^{P.} ± 0.72	18.70 ^{P..} ± 0.99

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. P < 0.05, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. .P < 0.05, significant at 5 % level.
..P < 0.01, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

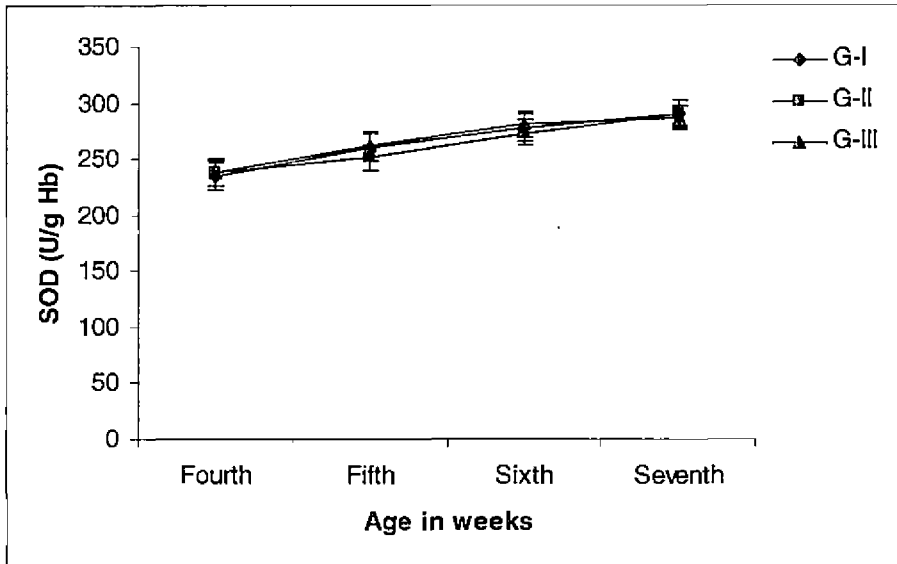


Fig. 19. Effect of acid silage (fish waste) and surimi waste powder on blood SOD level (U/g Hb) in broiler chicken

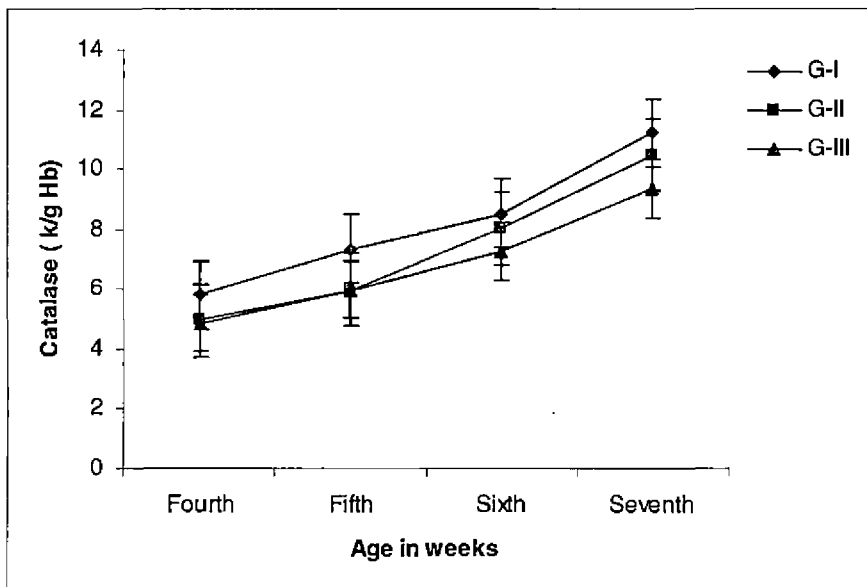


Fig. 20. Effect of acid silage (fish waste) and surimi waste powder on blood catalase level (k/g Hb) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

weeks. A significant ($P < 0.05$) increase was observed within group III between 5th and 6th weeks and within group I between 5th and 6th, 6th and 7th weeks of age. There was no significant ($P > 0.05$) difference between the three treatment groups at fourth, fifth, sixth and seventh weeks of age.

4.5.4.2. *Effect on catalase*

The values of catalase (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 12 and fig.20. The values in G-I ranged from 5.80 to 28.62 k/g Hb. The values in G-II ranged from 5.25 to 24.05 k/g Hb and in G-III ranged from 4.75 to 23.65 k/g Hb. There was no significant ($P > 0.05$) difference between the three treatment groups at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was observed within the groups I, II and III between 6th and 7th weeks of age and within group III between 4th and 5th weeks of age. There was a significant ($P < 0.05$) increase within the groups II and III between 5th and 6th weeks and within group I between 4th and 5th weeks of age.

4.5.4.3. *Effect on reduced glutathione (GSH)*

The values of GSH (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 13 and fig.21. The values in G-I ranged from 48.00 to 144.00 nmol/ml. The values in G-II ranged from 48.00 to 136.00 nmol/ml and in G-III ranged from 48.00 to 136.00 nmol/ml. There was no significant ($P > 0.05$) difference between the three treatment groups in the GSH value at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was noted within the three groups between the weeks 4th and 5th, 5th and 6th, 6th and 7th.

Table 13. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on reduced glutathione (GSH) and lipid peroxidation of broiler chicken from fourth to seventh week of age.

Parameter	GSH (nmol/ml) mean± S.E. (n = 15)				Lipid peroxidation (nmol/ml) mean± S.E. (n = 15)				
	Age (wks) Group	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G-I		54.12 ^P ± 0.42	62.52 ^{P..} ± 0.32	80.12 ^{P..} ± 0.60	128.92 ^{P..} ± 0.39	0.81 ^P ± 0.05	0.90 ^{P..} ± 0.04	1.01 ^{P..} ± 0.04	1.11 ^{P..} ± 0.05
G-II		52.12 ^P ± 0.18	59.08 ^{P..} ± 0.53	77.60 ^{P..} ± 0.37	127.60 ^{P..} ± 0.33	0.70 ^P ± 0.06	0.80 ^{P..} ± 0.06	0.94 ^{P..} ± 0.06	1.04 ^{P..} ± 0.06
G-III		53.20 ^P ± 0.20	64.80 ^{P..} ± 0.54	80.80 ^{P..} ± 0.56	129.60 ^{P..} ± 0.26	0.70 ^P ± 0.06	0.75 ^{P..} ± 0.06	0.84 ^{P..} ± 0.05	0.92 ^{P..} ± 0.06

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. P < 0.05, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. .P < 0.05, significant at 5 % level.
..P < 0.01, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

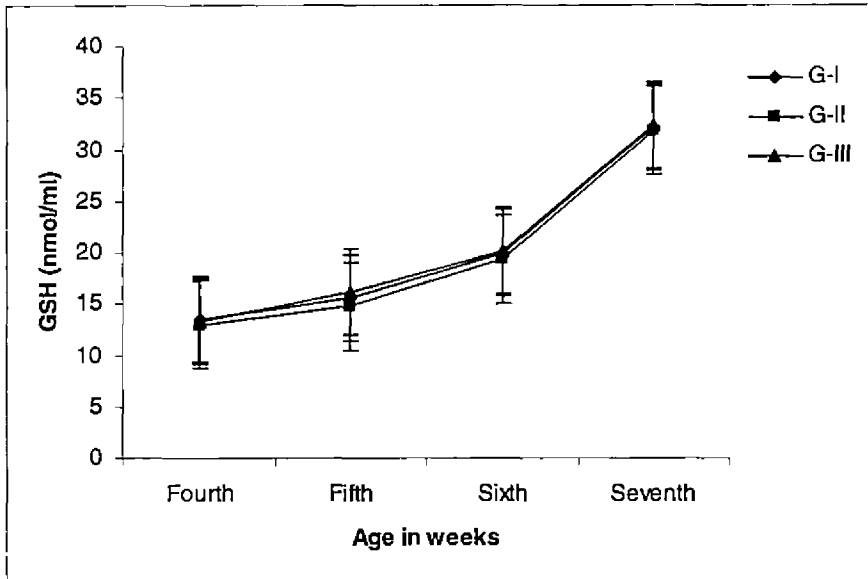


Fig. 21. Effect of acid silage (fish waste) and surimi waste powder on serum GSH level (nmol/ml) in broiler chicken

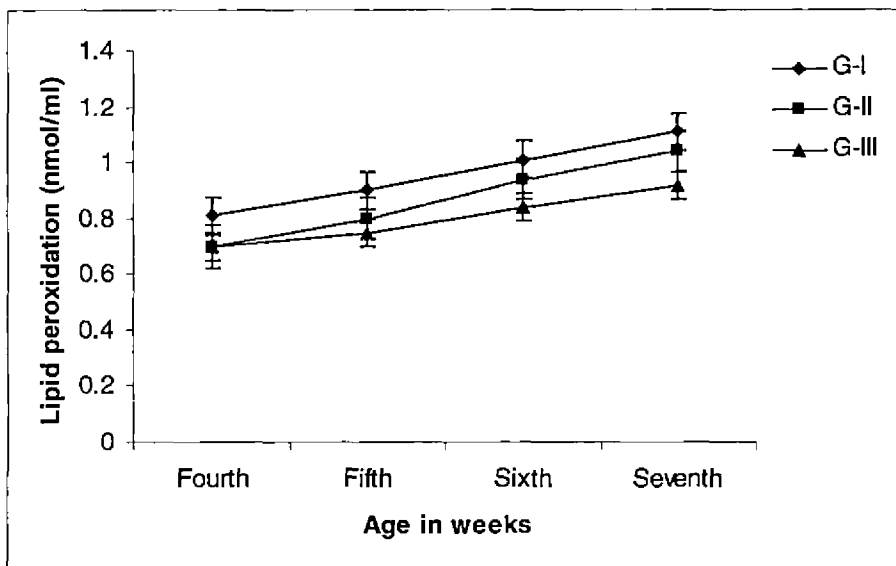


Fig. 22. Effect of acid silage (fish waste) and surimi waste powder on serum lipid peroxidation level (nmol/ml) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

4.5.4.4. *Effect on lipid peroxidation level*

The values of lipid peroxidation (mean \pm S.E.) at 4th, 5th, 6th and 7th weeks of age for the three treatments are given on table 13 and fig.22. The values in G-I ranged from 0.45 to 1.45 nmol/ml. The values in G-II ranged from 0.40 to 1.85 nmol/ml and in G-III ranged from 0.40 to 1.31 nmol/ml. There was no significant ($P > 0.05$) difference between the three groups at fourth, fifth, sixth and seventh weeks of age. There was a significant ($P < 0.01$) increase within the groups between 4th and 5th, 5th and 6th, 6th and 7th weeks.

4.5.5. **Effect on serum electrolytes:**

4.5.5.1. *Effect on sodium*

The values of sodium (mean \pm S.E.) at weekly intervals for the three treatments are on table 14 and fig.23. The values in G-I ranged from 63.25 to 161.37 mmol / l. The values in G-II ranged from 60.80 to 168.56 mmol/l and in G-III ranged from 62.45 to 178.00 mmol/l. There was no significant ($P > 0.05$) difference in the values between the three groups at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was observed within group I between 4th and 5th, 6th and 7th weeks; within group II between 5th and 6th weeks and within group III between 6th and 7th weeks of age. There was also a significant ($P < 0.05$) increase within group II between 6th and 7th of age.

Table 14. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum Na and K of broiler chicken from fourth to seventh week of age.

Parameter Age (wks) Group	Na (mmol/l) mean± S.E. (n = 15)				K (mmol/l) mean± S.E. (n = 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
G-I	117.12 ^P ± 5.84	123.30 ^{P..} ± 6.24	129.11 ^P ± 6.04	138.78 ^{P..} ± 6.70	3.11 ^P ± 0.16	3.24 ^{P.} ± 0.05	3.33 ^P ± 0.05	3.63 ^P ± 0.16
G-II	118.97 ^P ± 7.33	119.20 ^P ± 6.59	129.96 ^{P..} ± 6.72	139.21 ^{P.} ± 6.81	3.17 ^P ± 0.07	3.17 ^P ± 0.06	3.34 ^{P..} ± 0.07	3.44 ^{P.} ± 0.05
G-III	123.03 ^P ± 9.17	126.64 ^P ± 6.80	130.67 ^P ± 6.50	144.45 ^{P..} ± 6.23	3.26 ^P ± 0.04	3.37 ^P ± 0.06	3.39 ^P ± 0.06	3.51 ^{P..} ± 0.04

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. P < 0.05, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. [.]P < 0.05, significant at 5 % level.
^{..}P < 0.01, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

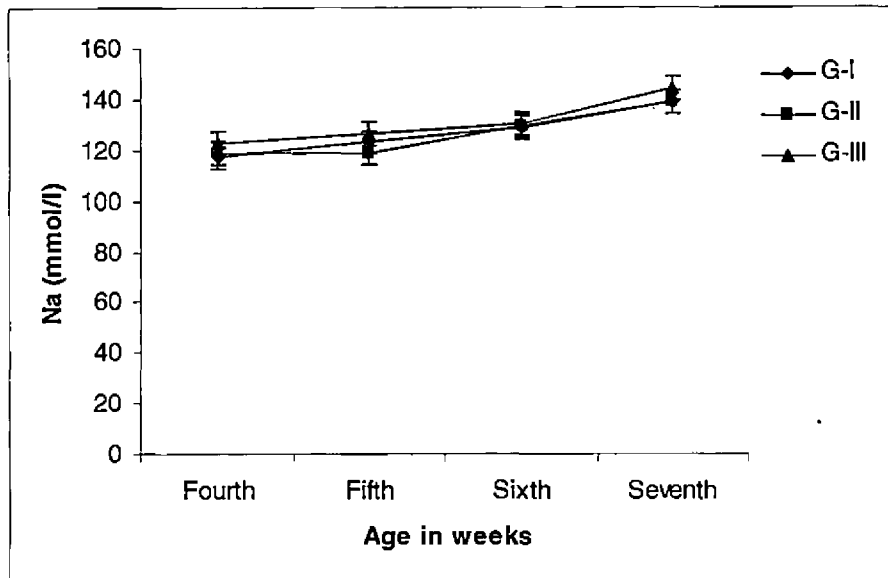


Fig. 23. Effect of acid silage (fish waste) and surimi waste powder on serum Na level (mmol/l) in broiler chicken

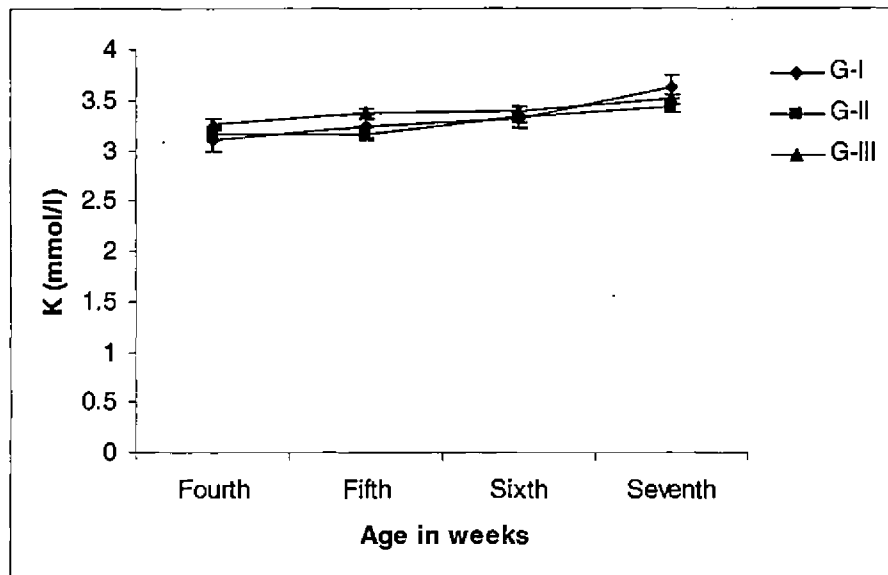


Fig. 24. Effect of acid silage (fish waste) and surimi waste powder on serum K level (mmol/l) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group

4.5.5.2. *Effect on potassium*

The values of potassium (mean \pm S.E.) at weekly intervals for the three treatments are on table 14 and fig.24. The values in G-I ranged from 2.70 to 5.70 mmol / l. The values in G-II ranged from 2.70 to 3.90 mmol/l and in G-III ranged from 3.10 to 3.90 mmol/l. No significant ($P > 0.05$) difference was observed between the three groups at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was observed within group II between 5th and 6th weeks and within group III between 6th and 7th weeks of age. There was a significant ($P < 0.05$) increase within G-I between 4th and 5th weeks of age and within G-II between 6th and 7th weeks of age.

4.5.5.3. *Effect on calcium*

The values of calcium (mean \pm S.E.) at weekly intervals for the three treatments are on table 15 and fig.25. The values in G-I ranged from 1.13 to 2.31 mmol / l. The values in G-II ranged from 1.21 to 2.32 mmol/l and in G-III ranged from 1.22 to 2.75 mmol/l. No significant ($P > 0.05$) increase was observed between the three groups at fourth, fifth, sixth and seventh weeks of age. A significant ($P < 0.01$) increase was noted within G-I, G-II and G-III between 4th and 5th, 6th and 7th weeks. A significant ($P < 0.05$) increase was observed within G-II and G-III between 5th and 6th weeks of age. Between 5th and 6th weeks, a significant ($P < 0.01$) increase was noted within group I.

Table 15. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on serum Ca, Mg and Fe of broiler chicken from fourth to seventh week of age.

Parameter	Ca (mmol/l) mean± S.E. (n = 15)				Mg (mmol/l) mean± S.E. (n = 15)				Fe (µmol/l) mean± S.E. (n = 15)			
	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh	Fourth	Fifth	Sixth	Seventh
Age (wks) Group												
G-I	1.44 ^P ± 0.05	1.63 ^{P..} ± 0.06	1.75 ^{P..} ± 0.06*	1.97 ^{P..} ± 0.05	0.81 ^P ± 0.02	0.82 ^P ± 0.008	0.97 ^{P..} ± 0.03	1.16 ^{P.} ± 0.05	29.36 ^P ± 0.74	32.10 ^{P..} ± 1.01	33.53 ^P ± 0.95	36.52 ^{P..} ± 1.18
G-II	1.45 ^P ± 0.03	1.61 ^{P..} ± 0.05	1.81 ^{P.} ± 0.06 ^{CP}	2.02 ^{P..} ± 0.05	0.72 ^P ± 0.01	0.90 ^{q..} ± 0.01	0.88 ^q ± 0.02	1.02 ^{P.} ± 0.05	29.95 ^P ± 0.96	32.70 ^{P..} ± 0.96	34.25 ^{P.} ± 0.97	35.92 ^{P.} ± 1.30
G-III	1.45 ^P ± 0.04	1.60 ^{P..} ± 0.04	1.79 ^{P.} ± 0.08 ^{CP}	1.93 ^{P..} ± 0.07	0.79 ^P ± 0.03	0.85 ^{Pq} ± 0.02	0.84 ^q ± 0.02	1.07 ^{P..} ± 0.04	31.39 ^P ± 1.10	32.22 ^P ± 1.05	34.25 ^{P.} ± 1.21	36.36 ^{P.} ± 1.18

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.

1. P < 0.05, significant at 5 % level.
In columns, means bearing same superscripts (p, q, r) do not differ significantly.
2. [.]P < 0.05, significant at 5 % level.
^{..}P < 0.01, significant at 1 % level.
In rows, means within groups were compared between subsequent weeks.

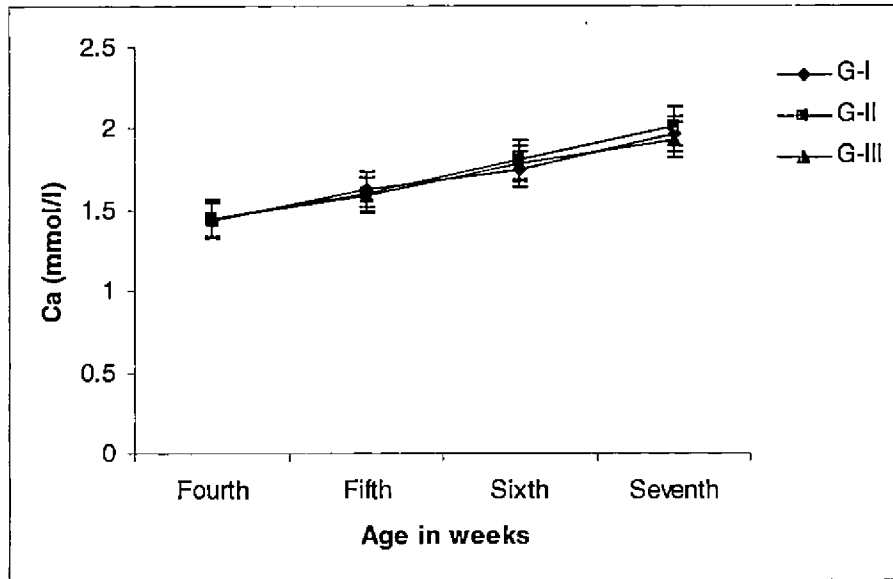


Fig. 25. Effect of acid silage (fish waste) and surimi waste powder on serum Ca level (mmol/l) in broiler chicken

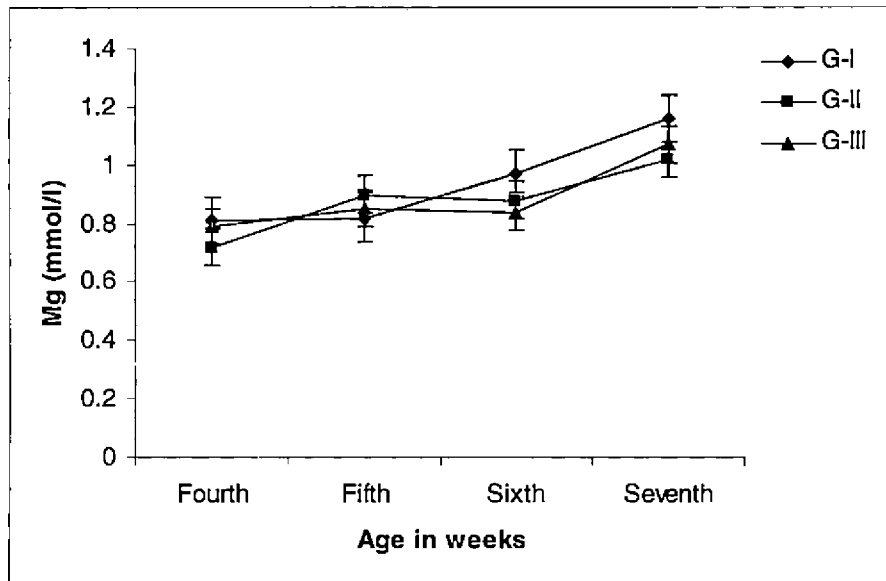
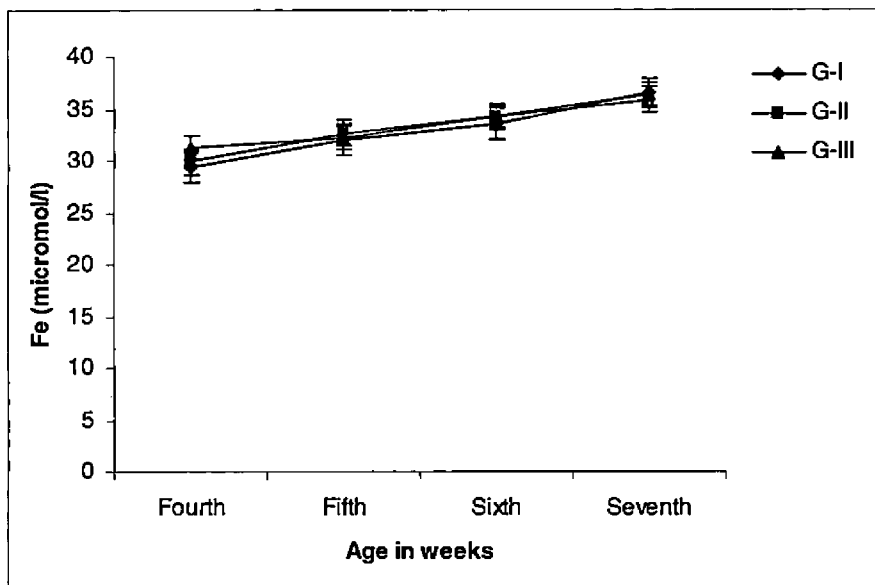


Fig. 26. Effect of acid silage (fish waste) and surimi waste powder on serum Mg level (mmol/l) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group, G-III – surimi waste powder fed group



**Fig. 27. Effect of acid silage (fish waste) and surimi waste powder on serum Fe level (micromol/l) in broiler chicken
G-I – control group, G-II – acid silage (fish waste) fed group,
G-III – surimi waste powder fed group**

4.5.5.4. *Effect of magnesium*

The values of magnesium (mean \pm S.E.) at weekly intervals for the three treatments are on table 15 and fig.26. The values in G-I ranged from 0.73 to 1.49 mmol / l. The values in G-II ranged from 0.61 to 1.31 mmol/l and in G-III ranged from 0.65 to 1.35 mmol/l. A significant ($P < 0.05$) increase was observed between the groups during 5th and 6th weeks. During 5th week, G-I was significantly ($P < 0.05$) different from G-II. During 6th week, G-I was significantly ($P < 0.05$) different from G-II and G-III. There was a significant ($P < 0.01$) increase within group I between 5th and 6th weeks; within group II between 4th and 5th weeks and within G-III between 6th and 7th weeks. There was a significant ($P < 0.05$) increase within G-II between 6th and 7th weeks.

4.5.5.5. *Effect of iron*

The values of iron (mean \pm S.E.) at weekly intervals for the three treatments are on table 15 and fig.27. The value in G-I ranged from 25.06 to 44.75 μ mol / l. The values in G-II ranged from 25.06 to 42.96 μ mol/l and in G-III ranged from 25.06 to 44.75 μ mol/l. A significant ($P < 0.01$) increase was observed within G-I and G-II between 4th and 5th weeks of age. Between 5th and 6th weeks, there was a significant ($P < 0.05$) increase within G-II and G-III. Between 6th and 7th weeks, a significant ($P < 0.05$) increase was noted within G-I and G-III. No significant ($P > 0.05$) increase was observed between the three groups at fourth, fifth, sixth and seventh weeks of age.

Table 16. Effect of dietary supplementation of acid silage (fish waste) and surimi waste powder on giblet weight, meat crude fat, meat total ash, meat crude protein (fresh basis) and meat cholesterol of broiler chicken at seventh week of age.

Parameter Group	Crude fat (g %) mean± S.E. (n = 3)	Total ash (g %) mean± S.E. (n = 3)	Crude protein (g %) mean± S.E. (n = 3)	Cholesterol (mg/dl) mean± S.E. (n = 3)
G-I	0.48 ^P ± 0.02	1.25 ^P ± 0.04	22.26 ^P ± 0.44	72.07 ^P ± 2.53
G-II	0.48 ^P ± 0.02	1.30 ^P ± 0.05	22.38 ^P ± 0.36	73.00 ^P ± 2.51
G-III	0.42 ^P ± 0.02	1.32 ^P ± 0.05	22.67 ^P ± 0.39	72.00 ^P ± 1.75

G-I- Control group; G-II- Acid silage (fish waste) fed group; G-III- Surimi waste powder fed group.
P < 0.05, significant at 5 % level.

In columns, means bearing same superscripts (p, q, r) do not differ significantly.

4.5.6. Effect on meat parameters (cholesterol, crude protein, ether extract, and total ash)

The mean values for meat parameters for the three treatments are given on table 16. There was no significant ($P > 0.05$) difference between the three treatment groups in the case of meat parameters like crude protein, ether extract, total ash and cholesterol. The values for crude protein were 19.08 g %, 24.63 g % and 23.07 g % for G-I, 19.95 g %, 23.15 g % and 24.04 g % for G-II and 24.15 g %, 23.77 g % and 20.09 g % for G-III and those of ether extract ranged were 0.50 g %, 0.52 g % and 0.42 g % for G-I, 0.38 g %, 0.51 g % and 0.55 g % for G-II and 0.36 g %, 0.53 g % and 0.37 g % for G-III. The values for total ash ranged from 1.02 g %, 1.52 g % and 1.21 g % for G-I, 1.23 g %, 1.05 g % and 1.62 g % for G-II and 1.48 g %, 1.65 g % and 0.83 g % for G-III. The cholesterol concentrations ranged from 55.00 to 74.00 mg / dl for G-I, 49.00 to 88.00 mg/dl for G-II and 63.00 to 85.00 mg/dl for G-III.

Discussion

5 DISCUSSION

5.1 BODY WEIGHT

Body weight is considered as an index of the nutritional status of an animal (Bhosale and Rao 2001)

According to Johnson *et al* (1985) the body weight of broiler male chicken of six weeks of age fed with standard ration was 1737 g but male chicken fed with 2.5 % 5 % and 10 % fish waste acid silage meal at the expense of soyabean meal acquired a body weight of 1883 1808 and 1864 g respectively. Chicken fed with 2.5 % 5 % and 10 % fermented fish waste silage meal at the expense of soyabean meal acquired a body weight of 1874 1838 and 1896 g respectively. The mean body weight obtained in the present experiment for the groups in which 10 % acid silage (fish waste) and surimi waste powder were added were 1503 and 1490 g respectively. Though there was no significant difference in the body weight with the incorporation of acid silage (fish waste) or surimi waste compared to those fed with unsalted dried fish, the higher body weight in the groups studied by Johnson *et al* (1985) might be due to the difference in sex species of bird used and type of feed. They incorporated silage prepared from filleting waste in the study using male birds. Male chicken normally gain more body weight than females under identical conditions (Peebles *et al* 1997a) but in the present experiment birds of either sex were used.

In the current study the body weights of the birds in the three groups were within the normal range and did not change significantly between the three groups. This was in agreement with Johnson *et al* (1985) who observed no change in body weight on inclusion of either acid silage meal or fermented silage meal up to a level of 10 % in the diet of broilers. The findings of the current study were also in

agreement with the reports of Espe *et al* (1992) who observed that four week old chicken fed with fish silage in graded amounts showed the same or better weight gains compared with those fed all dietary fish protein as fish meal

Hammoum *et al* (1998) reported that the broilers showed a net increase in live weight when one to two parts fermented fish silage of the total diet (four parts) was supplemented in the diet. This increase might be due to the difference in fish species (*Sardinia pilchardus*) and its processing methodology (*Lactobacillus plantarum*). The body weights of fourth to sixth week old normal control group of broiler chicken varied from 703 to 1480 g (Kanagaraju 1998) 1030 to 1832 g (Balasubramanian 2000) and 1012 to 1840 g (Anil 2001)

Ngoan *et al* (2001) observed that replacement of 50 % fish meal (FM) with ensiled shrimp by product (ESB) improved body weight gain in pigs. On using 100 % ESB body weight was found to be reduced due to low palatability of ESB. But no such partial replacement study was included in the present experiment. According to Kadari (2001) the body weight of normal broilers of fourth, sixth and eighth weeks of age were 924, 1616 and 2092 g respectively. Renjith (2004) observed that the body weights of normal control broiler chicken of four, six and eight weeks of age were 1118, 1974 and 2584 g respectively. Govindan (2005) observed reduction in body weight of layer ducks at 44 weeks of age by partial and complete replacement of dried fish with dried cuttle fish waste silage in layer ration. Lekshmy (2005) reported no change in the body weight of 26 weeks old Japanese quails where dried cuttle fish waste silage replaced fish meal in the ration. According to Ojewola *et al* (2005) the normal body weight of broiler chicken of Anak strain at seven weeks of age ranged from 1510 to 1878 g. The study conducted by Raghavan (2007) revealed that the feeding of dried fish or fermented fish waste silage of

Nemipterus japonicus did not have any adverse effect on the body weight of Japanese quails

Fasuyi (2007) observed that the body weight of normal control group of broiler chicken of fourth and sixth weeks of age were 787 g and 1260 g respectively. The control groups in the present experiment showed body weights at fourth and sixth weeks of age as 495 g and 1537 g respectively. There was not much difference between the control and experimental groups which showed that the nutritive values of experimental diets are similar to that of control diet which is of good quality and adequate to maintain normal growth and body weight of broiler chicken. Peebles *et al* (1997a) found that the body weight of male and female broiler chicken increased as age advanced. Fasuyi (2007) observed that body weight of birds of six weeks of age was higher than those of four weeks of age. An age related increase in body weight was also observed in the present investigation.

5.2 FEED CONSUMPTION

The quantity of feed consumed by birds reflects the health status of the birds and the palatability of the feed stuff used. In birds suffering from any ailment the appetite will be low and in turn feed consumption also will be reduced (Bhosale and Rao 2001).

Vizcarra Magana *et al* (1999) found that in three week old broiler chicken there was no difference in feed consumption between control group to which 0% fish silage was fed and experimental groups fed with 5%, 10% and 15% fish silage. The present findings were similar to the findings of Espe *et al* (1992) in four weeks old chicken who found no difference in feed intake in groups fed with 0, 5, 10, 20 and 30% fish silage. In pigs Ngoan *et al* (2001) found that daily feed intake was

reduced by the inclusion of shrimp by products in the diets and it might be due to palatability of the processed waste. The similar quantity of feed consumption in the three groups indicated that the incorporation of processed fish wastes does not affect the palatability and was accepted by the birds. The weekly feed consumption of broiler chicken from fifth to seventh weeks ranged from 819 to 899 g (Adeyemo and Longe 2007). The feed consumption from fifth to seventh weeks in the present investigation ranged from 1042 to 1315 g in control group of birds. There was not much variation in feed consumption between control group, acid sludge (fish waste) fed group and surimi waste fed group. This higher feed consumption in the present experiment might be due to higher palatability of the feed used. Govindan (2005) found that in layer ducks as age advanced there was an increase in daily feed intake in control group fed with diets containing dried fish and treatment groups fed with diets containing partial and complete replacement of dried fish with dried cuttle fish waste sludge. Talebal and Farzinpour (2006) observed that feed consumption increased as age advanced. The starter feed (upto three weeks of age) consumption was 1216 g while the grower feed (above three weeks upto six weeks of age) consumption was 2450 g. The feed consumed by the three groups during the entire study period was found to be similar and there was an increase in feed consumption within the groups as the age advanced.

5.3 GIBLET WEIGHT

The growth of the vital organs like heart and liver depends on the nutritional status of the birds (Bhosale and Rao 2001).

The giblet weight (combined weight of heart, liver and gizzard) of normal broiler chicken of eight weeks of age was 123 g (Kanagaraju 1998), 73.43 g (Kadam 2001) and 159g (Anil 2001). Balasubramanian (2000) reported the giblet

weight of six and eight week old broiler chicken as 111.94 and 159.58 g respectively. According to Renjith (2004) the mean gible weight of eight week old broiler chicken was 96.38 g. Ojewola *et al* (2005) observed that the mean gible weight for seven week old broiler chicken fed with locally processed fish waste meal was 88 g. Palod and Baghel (1998) observed that the mean value of gible weight in four week old broiler chicken fed with 5 % and 6 % fish meal protein was 51.24 g and 53.13g. The mean gible weight of birds in fish meal group, acid silage fed group and surimi waste fed group were 92 g, 88 g and 87 g respectively. The higher values obtained in the current study might be due to the increased per cent of fish protein (10 %) included in the diet. The non significant difference in gible weight observed between the three treatment groups at the end of the study revealed that the processed fish wastes in the diets contributed to the adequate growth of the vital organs.

5.4 HAEMATOLOGICAL PARAMETERS

Haemoglobin (Hb), Volume of Packed Red Cells (VPRC), Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) reflects the overall health status of an animal. When the protein quantity and quality of the feed are inadequate, it will depress the haematopoietic system leading to growth retardation and ill health (Stephenson 2002).

The normal Hb level in local chicken of four weeks of age was 7.73 g % (Islan *et al* 2004). Simi (2007) reported that the Hb level in normal six week old broiler chicken was 8.35 g %. According to Iheukwumere and Herbert (2003) Hb level in four week old broiler chicken was 13 g %. The Hb concentrations obtained in the present study ranged from 5.57 to 8.73 g % which concur with the above mentioned findings. The observed Hb concentrations in the present

experiment were similar in all the three groups and were within the normal range Islam *et al* (2004) reported that in local chicken Hb concentration increased with increase in age and a similar trend was observed in the present investigation

The VPRC of four week old normal broiler chicken was 38 % (Iheukwumere and Herbert 2003) Reddy (2003) found that the VPRC of four six and eight week old broiler chicken were 29.75 % 31.63 % and 29.63 % respectively According to Simi (2007) the VPRC of six week old normal broiler chicken was 26.88 % Islam *et al* (2004) observed that the VPRC of four week old local chicken was 27.73 % According to Peebles *et al* (1997) the VPRC of fourth and sixth week old normal broiler chicken were 26.50 % and 30.40 % respectively The VPRC in the present study ranged from 28 to 34 % which concu with the above mentioned findings The VPRC of acid silage (fish waste) fed group and surimi waste fed group were similar to that of the control group fed with unsalted dried fish Islam *et al* (2004) found that VPRC of local chicken increased as the age advanced According to Peebles *et al* (1997) haematocrit value increased in normal broiler chicken from two weeks to six weeks of age The VPRC values increased as the age advanced in the present study which was similar to the above mentioned findings

The mean TEC in five week old broilers was $2.01 \pm 0.08 (\times 10^6 / \mu\text{l})$ (Puvadolpirod and Thaxton 2000) According to Reddy (2003) the normal TEC in four week six week and eight week old broilers were 3.04 3.09 and 2.9 ($\times 10^6 / \mu\text{l}$) Islam *et al* (2004) observed that the mean TEC of four week old local chicken was $1.7 \times 10^6 / \mu\text{l}$ Simi (2007) observed that the mean normal TEC in six week old broilers was $2.97 \times 10^6 / \mu\text{l}$ In the present experiment TEC in the three groups were similar and ranged from 1.33 to 2.85 ($\times 10^6 / \mu\text{l}$) which concur with the above mentioned findings Within the groups the counts increased as the age advanced This is similar to the findings of Islam *et al* (2004) who reported that in chicken

TEC increased with increase in age According to Yohannan (2007) the TEC increased from fourth week to eighth week in normal broiler chicken

The normal TLC in five week old broiler chicken was $11.31 \times 10^3 / \mu\text{l}$ (Puvadolpirod and Thaxton 2000) Reddy (2003) observed that the mean TLC in fourth week to eighth week old broilers ranged from 23.74 to 26.49 ($\times 10^3 / \mu\text{l}$) According to Simi (2007) six week old broiler chicken had a TLC of $22.16 \times 10^3 / \mu\text{l}$ The TLC in the present study ranged from 15 to 25 ($\times 10^3 / \mu\text{l}$) which concur with the above mentioned findings In the present experiment there was no change in TLC between the three treatment groups and they all were in the normal range but as the age advanced the TLC was found to increase A similar age related increase was observed by Mashaly *et al* 2004 in broiler chicken

5.5 BIOCHEMICAL PARAMETERS

5.5.1 Serum protein profile

Serum proteins include albumin and globulin The serum protein level varies according to the protein quality of the diet The blood proteins maintain homeostasis regulate osmotic pressure and are involved in clotting mechanisms They carry several nutrients in the blood and are involved in immunological functions Protein deficiency in the diet causes depression in growth immunosuppression and increased susceptibility to diseases (Bhosale and Rao 2001)

Bowes *et al* (1989) observed that the total serum protein concentration in four week and six week old broiler chicken were 2.56 and 2.65 g/dl respectively The total plasma protein concentrations in normal broiler chicken of four, five and six weeks of age were 3.96, 4.05 and 4.08 g/dl respectively (Peebles *et al* 1997a) The

total plasma protein value in normal five week old broilers was 2.90 g/dl (Puvadolpirod and Thaxton 2000). The normal serum protein level in eight week old normal broiler chicken ranged from 3.30 to 4.97 g/dl (Kadar 2001, Anil 2001, Renjith 2004). According to Bunchasak *et al* (2005) the total serum protein concentration in normal laying hens at 33 weeks of age was 3.38 g/dl.

The serum protein level in normal eight week old broiler chicken ranged from 3.5 to 11.95 g/dl (Nworgu *et al* 2007, Adeyemo and Longe 2007). Emadi *et al* (2007) reported that the serum total protein concentration in five week and six week old normal broiler chicken were 4.50 and 4.24 g/dl and they noticed that there was no much change in the values as the age advanced. The serum total protein concentrations in the present study ranged from 1.67 to 3.05 g/dl which concur with the above mentioned findings. There was no change in the concentrations of total serum proteins between the control and experimental groups. As the age advanced there was an increase in the concentration of total proteins in all the three groups similar to the findings of Peebles *et al* (1997a).

According to Bowes *et al* (1989) the mean serum albumin concentrations in four week and six week old normal broiler chicken were 1.29 g/dl and 1.37 g/dl respectively. Emadi *et al* (2007) opined that the albumin concentration of five week and six week old normal broiler chicken were 1.50 and 1.52 g/dl respectively. The normal serum albumin level in eight week old broiler chicken was 2.10 g/dl (Nworgu *et al* 2007). Yohannan (2007) reported that the albumin concentration in four, six and eight week old normal control group of broiler chicken were 1.93, 1.96 and 1.87 g/dl respectively. These findings are similar to the present findings which ranged from 1.16 to 1.61 g/dl. There was no significant change in the concentration of albumin between the control and experimental groups during the entire study and the values were within the normal range. There was an increase in the values from

fourth to seventh weeks of age in all the three groups. Fanimo *et al* (2000) found that shrimp waste meal (SWM) fed rats were having lower albumin concentration than those fed with fish meal (FM) indicating that the protein quality affects the blood albumin concentration.

According to Bowes *et al* (1989) the concentration of globulin in broiler chicken of four week and six weeks of age were 2.15 and 1.89 g/dl respectively. The globulin concentration in eight week old normal broiler chicken was 1.4 g/dl (Nworgu *et al* 2007). Emadi *et al* (2007) observed that the concentration of globulin in five week and six week old broiler chicken were 3.00 and 2.72 g/dl respectively. Yohannan (2007) reported that the globulin concentration in four, six and eight week old broiler chicken were 3.01, 3.20 and 3.15 g/dl respectively. These findings are in accordance with the values in the present experiment which ranged from 0.8 to 1.45 g/dl. In the present study though the globulin concentration of the control group was significantly higher than that of the experimental groups at the start of the experiment, all the values were showing an increasing trend with increase in age. But at the end of the experiment (seventh week of age) the values were within the normal range and were not significantly different between the groups.

The albumin/globulin ratio in eight week old broiler chicken was 1.5 (Nworgu *et al* 2007). Yohannan (2007) observed that the A/G in four, six and eight week old broiler chicken were 0.64, 0.62 and 0.59 respectively. There was no significant change in A/G between the control and experimental groups and they were within the normal range. The A/G values ranged from 0.91 to 1.19 in the present study which concur with the above mentioned findings. In the group fed with surimi waste powder, there was a steady increase in the albumin/globulin ratio as the

age advanced but in the control group and the group fed with acid silage (fish waste) the ratio expressed a fluctuating trend

5.5.2 Serum enzymes

Aspartate amino transferase (AST) and alanine amino transferase (ALT) are two serum enzymes whose levels in the serum represent the normal functioning of organs like liver and muscle. In liver disorders, the concentrations of both ALT and AST in the serum increase. AST level is found to increase in the serum in heart diseases, muscular dystrophy, myositis, and acute pancreatitis. Increased serum amino transferase activity occurs in certain diseases involving tissues rich in these enzymes, notably the liver and myocardium, which is presumably due to the liberation of abnormally large amounts from the damaged tissues (Latner, 1975).

The AST value of four-week and six-week-old normal broilers ranged from 178 to 254 U/l (Bowes *et al.* 1989; Simi, 2007). Imaeda (1999) recorded the normal AST value in six-week-old normal broiler chicken as 128 U/l. The ALT value in four- to six-week-old normal broilers ranged from 6.50 to 22 U/l (Iheukwumere and Herbert, 2005; Simi, 2007). The AST value in fourth- to eighth-weeks of normal broiler chicken ranged from 195 to 211 U/l, and the values increased as the age advanced (Yohannan, 2007). The concentrations of ALT in the present experiment ranged from 10 to 19 U/l, and that of AST ranged from 130 to 182 U/l, respectively, and these values concur with the above-mentioned findings. In the present study, the AST and ALT values of the control group of birds were similar to those of acid silage (fish waste) and surimi waste powder-fed groups, and the values were found to increase as the age advanced and were within the normal range.

5.5.3 Serum lipid profile

Fats are the energy reserves present in animals. Cholesterol is the most important animal steroid from which other steroid compounds are formed. The level of cholesterol in blood is related to the development of atherosclerosis. The HDL level in serum is inversely related to the incidence of myocardial infarction. Non-esterified fatty acids (NEFA) is derived from lipolysis of triglyceride stored in adipose tissue. Serum NEFA is the portion of the total fatty acid pool that circulates in immediate readiness for metabolic needs. Knowledge of the level of NEFA can be helpful in the diagnosis and management of certain disorders of metabolism (Latner 1975).

5.5.3.1 Total lipids

Reddy (2003) observed that the value of plasma total lipids in normal broiler chicken from fourth to eighth weeks of age ranged from 532 to 614 mg/dl. Yohannan (2007) reported that the serum total lipid concentration ranged from 514 to 538 mg/dl in normal broiler chicken from fourth to eighth week of age. According to Simi (2007) the serum total lipid concentration in six week old broiler chicken was 714 mg/dl. The total lipid concentration of the control group was similar to that of acid silage (fish waste) fed group and surimi waste powder fed group. In the present study the concentrations of total lipids ranged from 376 to 754 mg/dl and they are similar to the above mentioned findings. According to Yohannan (2007) in broiler chicken the concentration of total lipids showed a fluctuating trend with the advancement of age from fourth to eighth week. But in the present experiment there was an increase in the concentration of total lipids as the age advanced in all the three groups throughout the study.

5.5.3.2 Triglycerides

According to Peebles *et al* (1997) the serum concentration of triglycerides in fourth, fifth and sixth week old normal broiler chicken were 122, 103 and 164 mg/dl respectively. The concentration of triglycerides in five week old normal control group of broiler chicken ranged from 82 to 95 mg/dl (Puvadolrod and Thaxton 2000, Kannan *et al* 2005). According to Reddy (2004) and Yohannan (2007) the plasma triglyceride concentration of normal control group of broiler chicken from fourth to eighth weeks of age ranged from 102 to 110 mg/dl. Emad *et al* (2007) reported that the serum triglyceride level in fifth and sixth week old normal control group of broilers were 106.60 and 102.40 mg/dl respectively.

Smitha (2005) reported that the triglyceride concentration of normal control group of broilers of six weeks of age was 124 mg/dl. The experimental groups were fed with rations in which protein in unsalted dried fish was replaced by protein from fermented fish waste sludge at 50 and 100 per cent levels. The 50 per cent replacement group showed a triglyceride concentration of 94 mg/dl while the 100 per cent replacement group showed 87 mg/dl but the triglyceride concentration was higher in the control group compared to the experimental groups. The concentrations of triglycerides in the three groups of the present experiment were comparable with the experimental groups of the study of Smitha (2005). In the present study, there was no difference in the concentration of triglycerides between the three groups of treatment.

According to the experiment conducted by Castillo *et al* (1999), supplementation of dietary fish oil to chicks reduced the level of triglycerides. Anil *et al* (1992) found that there was a decrease in triglyceride concentration in sardine

fed rats. In the present study, no such decrease in triglyceride concentration could be observed and it might be due to the use of fish wastes instead of fish and the difference in the species of fish used for the processing. The triglyceride concentrations in the present experiment ranged from 56 to 88 mg/dl and they are similar to the above mentioned findings. The concentration of triglycerides showed a fluctuating trend as the age advanced from fourth to eighth week of age in broiler chicken (Yohannan 2007) but in the present study the concentrations increased as the age advanced in all the three groups and were within the normal range.

5.5.3.3 High density lipoprotein (HDL)

Peebles *et al* (1997) reported that the serum HDL concentration in fourth, fifth, and sixth week old normal broilers were 88, 85, and 78 mg/dl respectively. The serum HDL concentration in five week old normal control group of broiler chicken ranged from 111 to 630 mg/dl (Puvadolrod and Thaxton 2000, Kannan *et al* 2005). According to Yohannan (2007), the plasma HDL concentration four, six, and eight week old normal control group of broiler chicken ranged from 41 to 52 mg/dl. The concentration of HDL in seven week old broiler chicken was 26 mg/dl (Anitha *et al* 2007). Emadi *et al* (2007) reported that the serum HDL level in fifth and sixth week old normal control group of broilers were 165 and 147 mg/dl respectively. During seventh week of age, there was a significant reduction in HDL concentration in acid silage (fish waste) fed group than that of control group of birds. This reduction is similar to the findings of Castillo *et al* (1999) who observed that in chicks, dietary fish oil supplementation resulted in reduction in HDL. Yohannan (2007) observed a fluctuation in the values as the age advanced from fourth to sixth weeks of age in normal broiler chicken. The mean concentrations of HDL in the present experiment ranged from 31 to 62 mg/dl and are supported by the findings

mentioned above. The HDL concentrations were found to be increasing with the advancement of age in all the three groups and were within the normal range.

5.5.3.4 Very low density lipoproteins (VLDL)

The VLDL concentrations from fourth to sixth week old broiler chicken ranged from 16.50 to 33 mg/dl (Peebles *et al.* 1997, Kannan *et al.* 2005). According to Yohannan (2007), the VLDL concentration of fourth, fifth and sixth week old normal broiler chickens were 21.77, 21.94 and 21.72 mg/dl respectively. Emadi *et al.* (2007) reported the serum VLDL level in fifth and sixth week old broiler chicken as 23.60 and 20.80 mg/dl respectively. As the age advanced, a fluctuating trend was noticed from fourth to eighth weeks of age in broiler chicken (Yohannan 2007). Anil *et al.* (1992) and Castillo *et al.* (1999) reported that the VLDL concentration decreased when fish oil was fed to rats and chicks respectively. No such reduction could be observed in the present study and this might be due to the difference in the species of fish and use of fish waste instead of fish oil. The mean concentrations of VLDL ranged from 11.23 to 17.61 mg/dl in the present study, which are similar to the above findings. The VLDL concentration in the control group of birds was similar to that of acid silage (fish silage) fed and surimi waste powder fed groups throughout the study. There was an increase in concentration of VLDL as the age advanced and all the values were within the normal range.

5.5.3.5 Non esterified fatty acids (NEFA)

The NEFA concentration of nine week old normal broiler chicken was 520 $\mu\text{mol/l}$ (Sands and Smith 2002). Newman *et al.* (2002) opined that the mean value of plasma NEFA for five week old normal broiler chicken fed with fish oil was 200 $\mu\text{mol/l}$. The concentration of NEFA in four week old broiler chicken was

206 $\mu\text{mol/l}$ (Ashwell and McMurty 2003) The mean NEFA concentrations in the present study ranged from 297 to 590 $\mu\text{mol/l}$ which concur with the above findings. The concentration of NEFA in the control group fed with unsalted dried fish and experimental groups fed with acid sludge (fish waste) and surimi waste powder were similar during the entire study period and as the age advanced the concentration increased.

5.5.3.6 Total cholesterol

Bowes *et al* (1989) observed that the serum total cholesterol in normal broiler chicken of fourth and sixth weeks of age were 4.23 and 3.23 mmol/l respectively. The total serum cholesterol of four week, five week and six week old normal broiler chicken were 122, 120 and 115 mg/dl respectively (Peebles *et al* 1997). Razdan *et al* (1997) reported that the total plasma cholesterol in two week old normal group of broiler chicken was 7.78 mmol/l. Newmann *et al* (2002) observed that the plasma cholesterol in five week old normal broiler chicken was 2.40 mmol/l. The concentration of total serum cholesterol in five week old normal group of broiler chicken ranged from 87 to 184 mg/dl (Puvadolpirod and Thaxton 2000, Kannan *et al* 2005). The total serum cholesterol concentration of six week old normal group of broiler chicken ranged from 149 to 176 mg/dl (Francis 2005, Simi 2007). Dhansing (2006) reported that the total serum cholesterol concentration in four week and six week old normal broiler chicken were 161 and 182 mg/dl respectively. Nwojgu *et al* (2007) reported that the total serum concentration in eight week old normal group of broilers was 143 mg/dl. Anitha *et al* (2007) observed that the concentration of total serum cholesterol in normal group of broiler chicken of seven weeks of age was 91 mg/dl. Yohannan (2007) reported that the total plasma cholesterol in normal broiler chicken of fourth to eighth weeks of age ranged from 129 to 136 mg/dl. Emad *et al* (2007) reported that the total serum cholesterol

in normal broiler chicken at fifth and sixth weeks of age were 132 and 122 mg/dl respectively. Dhansing (2006) observed that there was an increase in the serum cholesterol concentration as the age advanced. The mean serum cholesterol values obtained in the present experiment ranged from 68 to 104 mg/dl which were within the normal range according to the above mentioned studies. In the present experiment the observed values for total serum cholesterol in the control and experimental groups were similar throughout the study and as the age advanced there was an increase in the concentration in all the three groups.

5.5.4 Antioxidant status

Antioxidants help to stop cell destruction caused by free radicals and are considered to be the scavengers of free radicals. The body's ability to produce antioxidant enzymes can be hampered by improper nutrition (Latner 1975).

The concentrations of SOD in normal chicken in seventh to eighth week old broiler chicken ranged from 260 to 295 U/g Hb (Reddy *et al.* 2004, Ramnath *et al.* 2007). In the present experiment the observed mean values in the three groups at the end of the study (seventh week) ranged from 287 to 291 U/g Hb which were concurrent with the above study. The GSH concentrations in seventh to eighth week old chicken ranged from 50.70 to 144.88 nmol/ml (Reddy *et al.* 2004, Ramnath *et al.* 2007) which were similar to the observed values in the present study and the values ranged from 52 to 130 nmol/ml. The values obtained for lipid peroxidation in the present study (0.70 to 1.11 nmol/ml) were slightly lower than that was observed by Reddy *et al.* (2004), Ramnath *et al.* (2007) and Yohannan (2007) (1.80 to 3.65 nmol/ml) but there was no significant difference among the birds treated with fish meal and silage (fish waste) and surimi waste powder. The concentrations of catalase in normal chicken in fourth to eighth week old broiler

chicken ranged from 2.46 to 24.04 k/g Hb (Reddy *et al.* 2004, Ramnath *et al.* 2007, Yohannan 2007). The mean values of catalase in the present experiment in the three treatment groups ranged from 9.74 to 22.44 k/g Hb which were similar to the above mentioned findings. There was no change in the values of SOD, catalase, GSH and LPO between the control and experimental groups. Yohannan (2007) observed a fluctuating trend in the values of SOD, catalase and LPO with the advancement of age. But in the present experiment with the advancement of age the values of the four parameters increased.

5.5.5 Serum electrolytes

Minerals are the inorganic constituents of body tissue. They constitute around four per cent of the body weight. They are the structural components of the body and maintain acid base balance. They act as catalysts in enzyme and hormonal functions. Deficiency of calcium results in loss of appetite and weakness whereas magnesium deficiency results in anorexia, depressed growth and muscular incoordination. Iron deficiency results in low growth rate and anemia. Na and K deficiency result in reduced appetite and growth retardation (Bhosale and Rao 2001).

The serum sodium concentrations in fourth to eighth week old normal broiler chicken ranged from 103 to 158 mmol/l (Bowes *et al.* 1989, Imaeda 1999, Francis 2005, Nworgu *et al.* 2007). The serum sodium concentrations of the three groups in the present experiment were similar to these findings and it ranged from 117 to 144 mmol/l. The serum potassium concentrations in fourth to eighth week old normal broiler chicken ranged from 2.40 to 5.8 mmol/l (Bowes *et al.* 1989, Imaeda 1999, Francis 2005, Nworgu *et al.* 2007). The serum calcium concentration in normal broiler chicken of fourth week to eighth week of age ranged from 1.62 to 3.01 mmol/l (Bowes *et al.* 1989, Kanagaraju 1998, Balasubramanian 2000).

Kollanoor 2004 Francis 2005 Kroliczewska and Zawadski 2005) These findings concur with the findings in present study that ranged from 1.44 to 2.02 mmol/l. The serum magnesium concentrations in the present experiment ranged from 0.72 to 0.97 mmol/l. These findings concur with the findings of (Bowes *et al* 1989 Kollanoor 2004). In their study the magnesium concentration for normal broiler chicken from fourth to eighth week of age ranged from 0.74 to 1.09 mmol/l. The mean serum iron concentration in six week old normal broiler chicken ranged from 18.63 to 58.17 $\mu\text{mol/l}$ ((Kroliczewska and Zawadski 2005 Kollanoor 2004) and they were similar to the findings in the present experiment (29 to 36 $\mu\text{mol/l}$).

Broiler chicken (six week old) fed rations having unsalted dried fish and unsalted dried fish replaced with fermented fish silage at 50 % and 100 % levels had serum calcium levels of 2.43 mmol/l, 2.60 mmol/l and 2.35 mmol/l respectively (Smitha 2005) which concur with the present findings.

The concentrations of Na, K, Ca and Fe were similar in the control and experimental groups. Moniello *et al* (2005) reported that in ostriches the serum K, Ca and Fe concentrations increased with the advancement of age. In the present study as the age advanced the concentrations of Na, K, Ca and Fe were found to increase in all the three groups.

Even though during the fifth week the concentration of serum Mg in acid silage (fish waste) fed group was higher than that of the control group fed with unsalted dried fish, at the end of the study there was no significant difference between the three treatment groups. There was an increase in the magnesium concentration as the age advanced in all the three groups.

5.5.6 Meat parameters (crude protein, ether extract, total ash, cholesterol)

Broiler chicken are meant for meat. High cholesterol containing meat is not preferred for human consumption. The crude protein, ether extract and total ash are determined to assess the nutritional quality of poultry meat (Baeza, 2004).

The crude protein, ether extract and ash in the meat of seven week old broiler chicken fed with locally processed fish waste meal were 386.90, 63.40 and 87.30 g / kg DM respectively (Ojewola *et al.* 2005). The values of crude protein in raw broiler chicken meat were ranging from 21.39 to 21.99 g % (Stadelman *et al.* 1988, Dhansing 2006) which were concurrent with the mean values in the present study (22.26 to 22.67 g %). The mean values of ether extract obtained in the present experiment ranged from 0.42 to 0.48 g %. Similar findings were seen in the studies of Kalavathy *et al.* (2006) and (Stadelman *et al.* 1988) where the values ranged from 0.89 to 1.65 g %. The total ash in raw broiler chicken meat was 0.98 g /100 g edible portion according to Stadelman *et al.* (1988) and the mean value in the present study was between 1.25 and 1.32 g %. The mean cholesterol values in the present experiment ranged from 72 to 73 mg/dl. The findings were similar to the observations of Stadelman *et al.* (1988), Skrivan *et al.* (2002), Kalavathy *et al.* (2006), Dhansing (2006) and Anitha *et al.* (2007) where cholesterol value ranged from 54 to 87 mg/dl. The values of crude protein, ether extract, total ash and cholesterol in the control group of birds were similar to the groups fed acid silage (fish waste) and surimi waste powder.

SUMMARY

6 SUMMARY

The present study was undertaken to evaluate the effect of dietary incorporation of processed fish wastes namely acid silage (fish waste) and surimi waste powder on the physiological and biochemical parameters in broiler chicken.

The investigation was conducted in forty five broiler chicken of Vencob strain. They were given standard broiler starter ration of BIS specification for the first three weeks. After three weeks of age they were randomly divided into three groups (GI, GII and GIII) of 15 birds in each group and the study was conducted from four weeks to seven weeks of age. GI served as the normal control group and was fed with the standard broiler finisher ration. GII was fed with the standard broiler finisher ration for four weeks in which unsalted dried fish was completely replaced with acid silage (fish waste) and the feed was made isocaloric and isonitrogenous. GIII was fed with the standard broiler finisher ration for four weeks in which unsalted dried fish was completely replaced with surimi waste powder and the feed was made isocaloric and isonitrogenous.

The body weight and feed consumption of the birds in each group were recorded at weekly intervals from fourth week till the end of experiment (seventh week). Blood samples were collected with and without anticoagulant (heparin) from fourth week to seventh week at weekly intervals for the estimation of hematological and biochemical parameters. Hematological parameters such as Haemoglobin (Hb), Volume of Packed Red Cells (VPRC), TEC and TLC were estimated. Biochemical parameters like serum total proteins, serum albumin, serum globulin, albumin/globulin ratio, serum total lipids, serum triglycerides, serum High Density Lipoproteins (HDL), Very low Density Lipoproteins (VLDL), serum non esterified fatty acids, serum total cholesterol, antioxidants like blood superoxide dismutase (SOD), blood catalase, serum reduced glutathione (GSH), serum lipid peroxidation

level (LPO) serum enzymes like ALT AST serum minerals like Na K Ca Mg and Fe were also analyzed. The quality of the meat was also assessed by the estimation of meat parameters like cholesterol crude protein ether extract and total ash from three representative samples from each group. The weight of giblet was also determined at the end of the study.

All the three groups showed an increase in body weight and feed consumption with the advancement of age. There was no significant difference in body weight between the groups and they maintained normal weight gain. No significant difference was observed in the giblet weight between the control and experimental groups indicating proper growth of internal organs.

All the haematological parameters (Hb VPRC TEC and TLC) maintained an increasing trend as the age advanced in the three groups and they were within the normal range. The values of the control and experimental group were statistically similar indicating that they support normal haematopoiesis and production of blood cells.

The serum total protein and albumin values did not express any significant difference between the control and experimental groups. The values increased with increase in age and were within the normal range. Although the globulin concentration of the control group was significantly higher than the experimental groups at the start of the experiment, all the values showed an increasing trend with increase in age. But at the end of the experiment, the values were within the normal range and were not significantly different between the groups. There was no significant difference in albumin globulin ratio between the control and experimental groups and they were within the normal range. In the group fed with surimi waste powder, there was a steady increase in the albumin globulin ratio as the age

advanced but in the control group and the group fed with acid silage (fish waste) the ratio expressed a fluctuating trend. Thus the protein requirement and synthesis of the serum proteins are adequately met with the processed fish wastes.

An age related increase was observed in the concentrations of ALT and AST. The concentrations were not significantly different between the three groups and they were in the normal range indicating that no deleterious effect is induced by the replaced fish wastes.

The concentrations of total lipids, triglycerides, HDL, VLDL, NEFA and total cholesterol were found to increase from fourth week till seventh week and they were within the normal range. There was no significant difference between the groups in lipid profile throughout the study except in the case of HDL concentration where a significant reduction was observed in acid silage (fish waste) fed group compared to control group at the end of the study. The present study indicates that the lipid metabolism is adequately supported by the replaced processed fish wastes.

The concentrations of SOD, catalase, GSH and LPO were similar in the control and experimental groups which proved that the antioxidant status was normal in the experimental groups even though the ration included fish wastes instead of fish. An age related increase was found with all the values. This indicated that the replaced fish wastes do not depress the antioxidant status of the birds.

The meat cholesterol concentration of the groups fed with acid silage (fish waste) and surimi waste powder were not significantly different from that of the control group fed with unsalted dried fish which proved to be advantageous. The proximate analysis of the meat revealed that the crude protein, ether extract and total

ash of the experimental groups were similar to that of control group indicating that meat quality is maintained with these unconventional protein sources

There was no significant difference in the serum sodium potassium calcium and iron content among the three groups. At fifth week of age the magnesium level was significantly higher in acid silage group while at sixth week of age the birds fed with processed fish wastes were having significantly lower level than the control group but finally at the end of the experiment (seventh week) there was no significant difference between any of the groups

It is obligatory to use animal protein sources in broiler chicken ration for better growth performance. Fish meal is the normal ingredient in the ration since it is the source for the amino acids of animal protein origin like lysine and methionine that are deficient in the vegetable protein supplements but highly essential for proper growth and development of chicken. But due to the high cost and low availability of fish meal unconventional sources like fish wastes are being tried as alternative source of animal protein. In the present study physio-biochemical effects of using processed fish wastes in the ration of broiler chicken instead of fish meal were assessed. They induced a growth similar to that of the standard normal ration which contained fish meal as animal protein. Thus the incorporation of acid silage (fish waste) or surm waste powder could be advocated to the farmers to reduce the feed cost to improve the profit without affecting the growth and meat quality of broiler chicken and also as a measure to reduce environmental pollution.

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PHYSIO-BIOCHEMICAL EVALUATION OF BROILER CHICKEN FED WITH PROCESSED FISH WASTES

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ABSTRACT

The present study was undertaken to study the effect of dietary incorporation of processed fish wastes acid silage (fish waste) and surimi waste powder on the physio biochemical parameters in broiler chicken

The experiment was conducted on forty five day old broiler chicken of Vencob strain for a period of seven weeks. After three weeks of age they were randomly divided into three groups G I G II and G III of 15 birds each. During the first three weeks they were fed with standard broiler starter ration of BIS specification. After three weeks G I was fed with the standard broiler finisher ration G II was fed with the standard broiler finisher ration in which there was 100 per cent replacement of unsalted dried fish with acid silage (fish waste) and G III was fed with the standard broiler finisher ration in which there was 100 per cent replacement of unsalted dried fish with surimi waste powder. The rations of G I G II and G III were made isocaloric and isonitrogenous.

Body weight and feed consumption were recorded at weekly intervals from fourth to seventh weeks of age. Blood samples were collected fourth to seventh week from the three groups at weekly intervals and haematological and biochemical parameters were analysed. Haematological parameters like Hb VPC TEC TLC serum protein profile like total protein albumin globulin albumin globulin ratio serum lipid profile like total lipids triglycerides HDL VLDL NEFA total cholesterol serum enzymes like ALT AST antioxidants like blood catalase blood SOD serum GSH serum LPO serum electrolytes like Na K Ca Mg Fe were estimated. At the end of the experiment the birds were sacrificed and giblet weight was assessed. The meat of three representative samples from each group was used for analysing meat cholesterol and the proximate principles like crude protein ether extract and total ash.



There was no significant difference between the three groups in body weight and feed consumption and they were gradually increasing during the experimental period in all the three groups. The haematological parameters also were comparable between the control and experimental groups. No significant difference was observed between the groups in the concentrations of total protein, albumin and albumin globulin ratio throughout the study. The concentration of globulin was significantly higher in the control group at the start of the study but later the values were significantly comparable between the three groups. The concentrations of total lipids, triglycerides, NEFA, VLDL and total cholesterol maintained a similar trend between the control and experimental groups but at the seventh week the concentration of HDL in the acid silage (fish waste) fed group was lower than that of the control group. The concentrations of serum enzymes and minerals (Na, K, Ca and Fe) did not show any significant change between the three groups during the entire study. Though the Mg level showed a fluctuating trend at fifth and sixth weeks of age, at the end of the study there was no significant difference between any of the groups. The antioxidant status was also significantly comparable between the groups. The meat parameters like g/bet weight, meat cholesterol and the proximate principles were also significantly similar in the three groups.

It is obligatory to use animal protein source in broiler chicken for obtaining better growth performance. Fish meal is rich in certain amino acids essential for the proper growth of chicken that are limiting in the vegetable protein supplements. Unconventional protein sources like fish wastes are to be used in the ration due to the high cost and low availability of fish meal. In the present study, physiological/biochemical effects of using processed fish wastes in the ration of broiler chicken instead of fish meal were assessed and they induced a growth similar to that of the standard normal ration which contains fish meal as animal protein. Thus the incorporation of the two fish wastes could be advocated to the farmers to reduce the feed cost and improve the profit without affecting the growth and meat quality of broiler chicken and also as a measure to minimize environmental pollution.