EFFECT OF REPLACING FISH MEAL PROTEIN BY SHRIMP WASTE MEAL PROTEIN WITH OR WITH OUT AMINOACIDS ON PERFORMANCE OF BROILERS

By

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This is to certify that the thesis entitled **"EFFECT OF REPLACING FISH MEAL PROTEIN BY SHRIMP WASTE MEAL PROTEIN WITH OR WITH OUT AMINOACIDS ON PERFORMANCE OF BROILERS"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** of the **SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI,** is a record of bonafide research work carried out by **Miss. N.MOUNICA**, **TVM/2014-29** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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I, **N.MOUNICA, ID NO: TVM/2014-29** hereby declare that the thesis entitled **"EFFECT OF REPLACING FISH MEAL PROTEIN BY SHRIMP WASTE MEAL PROTEIN WITH OR WITH OUT AMINOACIDS ON PERFORMANCE OF BROILERS"** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **MASTER OF VETERINARY SCIENCE** is the result of original research work done by me. It is further declared that the thesis or any part there of has not been published earlier in any manner.

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(Mounica)

LIST OF ABBREVIATIONS

%	:	Percent
AIA	:	Acid insoluble ash
AOAC	:	Association of Official Analytical Chemists
Avg	:	Average
BIS	:	Bureau of Indian Standards
BWG	:	Body weight gain
Ca	:	Calcium
CF	:	Crude fibre
Cfu	:	Colony forming unit
COS	:	Chito –oligosaccharides
CP	:	Crude protein
Dl	:	Decilitre
DM	:	Dry matter
DMB	:	Dry matter basis
DORB	:	De oiled rice bran
EE	:	Ether extract
FCR	:	Feed conversion ratio
FE	:	Feed efficiency
FI	:	Feed intake
Fig	:	Figure
FM	:	Fish meal
Gm	:	Grams

ICAR	:	Indian Council for Agricultural Research
Kg	:	Kilo grams
Kcal	:	Kilo calories
ME	:	Metabolizable energy
mg	:	Milligram
ml	:	Milli litres
ul	:	Microlitre
NRC	:	National Research Council
NFE	:	Nitrogen free extract
NS	:	Non-significant
NA	:	Not available
P	:	Phosphorus
Rs	:	Rupees
RBC	:	Red blood cell
WBC	:	White blood cell
SBM	:	Soya bean meal
SPC	:	Standard plate count
SWH	:	Shrimp waste hydrolysate
SWM	:	Shrimp waste meal
TA	:	Total ash
TME	:	True metabolisable energy
i.e	:	That is
Wt	:	weight

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ABSTRACT

The present study was carried out with an objective to investigate the growth performance, digestibility of nutrients, gut pathogen population, serological parameters, haematological parameters and carcass traits of broilers fed diets containing shrimp waste meal with or with-out amino acids.

Basal pre-starter, starter and finisher broiler diets were formulated with conventional feed ingredients like maize, soya bean meal, de-oiled rice bran (DORB) and fish meal as per the Nutrient Requirements of Poultry (ICAR,

2013). The CP (%) and ME (kcal/kg) contents of the basal pre starter, starter and finisher diets were 22.04, 3000; 21.50, 3049 and 19.57, 3099 respectively.

The experimental diets in pre-starter phase were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 20% level (T_2) , 30% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. In starter and finisher phases five experimental diets were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 50% level (T_2) , 100% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB and 10 % fish meal. All diets were iso-nitrogenous and iso-caloric.

Three hundred and seventy five day old commercial broiler chicks were distributed randomly to five treatments with three replicates of twenty five birds each. The experiment was carried out from day old to 42 days of age. Weekly body weight gains and feed intake were recorded and feed efficiency was calculated accordingly. Metabolism trials were conducted to study the digestibility of nutrients during starter (0-28 days) and finisher (29-42 days) phases. At the end of 2, 4 and 6 weeks two birds per replicate and thus a total of six birds per treatment were sacrificed to study the effect of shrimp waste meal on *E.coli* and *Salmonella* count of the cecal contents and on serological, haematological parameters. Carcass traits were studied at the end of the experimental trial.

There was no significant difference in body weight gain among treatments during pre-starter phase.The body weight gain (BWG) was found significantly ($P<0.01$) higher in birds fed (T_4, T_2 and T_1) diets when compared to birds fed T_3 and T_5 during starter, finisher and overall phases. Feed intake was found to be significantly ($P<0.01$) higher in birds fed with control diet compared to birds fed with (T_2 to T_5) diets at pre-starter phase. During starter, finisher and overall growth phases no significant difference was noticed regarding feed intake among treatments. Irrespective of the growth phase the feed conversion ratio (FCR) was found non significant among treatments.

The nutrient digestibility of DM and CF was found to be non-significant among treatments during starter and finisher phase. However during starter and finisher phases CP and EE digestibilities were found significantly ($P<0.01$) higher in birds fed T_4 diet when compared to birds fed other diets T_1, T_2, T_3 and T_5.

The *E.coli* and *Salmonella* count (log cfu/g) of cecal contents were found significantly ($P<0.01$) lower in birds fed SWM supplemented diets T_2,T_3, T_4 and T_5 than control diet (T_1) during pre-starter, starter and finisher phases.

In pre-starter and starter phases no significant difference was noticed regarding levels of serum total protein (g/dl), albumin (g/dl), globulin (g/dl), glucose (mg/dl) and cholesterol (mg/dl) among treatments (T_1 to T_5). In finisher phase also there was no significant difference in levels of serum total protein (g/dl), albumin (g/dl) and glucose (mg/dl) among treatments, but the serum cholesterol levels (mg/dl) and globulin levels (g/dl) were found significantly ($P<0.01$) higher in birds fed T_1 diet than birds fed other diets (T_2, T_3, T_4 and T_5).

Non significant differences were noticed among treatment groups regarding RBC count during the three phases of the study. During pre-starter phase the WBC count ($10^3/\mu l$) was significantly higher ($P<0.01$) in the birds fed with the diets T_2, T_3 ,T_4 and T_5 when compared to the birds fed with T_1(control diet). During starter and finisher phases the WBC count ($10^3/\mu l$) was significantly higher ($P<0.01$) in the birds fed with the diets T_2 and T_3 than the birds fed with T_1 , T_4 and T_5. During the pre-starter and starter phases lymphocyte (%) count was significantly higher ($P<0.01$) in T_2 and T_3 than in T_1, T_4,and T_5 fed birds.Whereas during the finisher phase there was no significant difference among treatments.

At the end of experimental period, the live weight gain, hot carcass weight and the dressing percentage were found significantly ($P<0.01$) higher in birds fed T_4 diet when compared to birds fed other diets. The liver, gizzard and heart weights (g) were found significantly ($P<0.01$) higher in birds fed T_4, T_2, and T_1 diets when compared to T_3 and T_5 group.

The results of the present study indicated that the Protein from FM can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets for good economic returns and productive performance with amino acid supplementation (lysine and methionine).

CHAPTER- I

INTRODUCTION

The Poultry Industry has emerged as the fastest growing segment of the livestock sector globally due to a number of favorable reasons. Poultry sector, besides providing direct or indirect employment to people, is also a potential tool for ameliorating poverty for many landless and marginal farmers. In addition to contributing to improved human nutrition and food security by being a leading source of high quality protein, poultry/chicken is of economic, social and cultural significance in small societies (FAO, 2010).

Indian Poultry Industry ranks 3rd in egg production and 5th in broiler production (USDA, 2013). Poultry Industry has however been confronted with the challenges of high cost and scarcity of feed inputs. Feed cost ranges from 70-75 % of total cost of production (Sharifi *et al*., 2012). This arises from the direct competition between man and livestock. The protein component cost of broiler ration is high. Formulating poultry diets based on conventional feed stuffs such as fish meal and soybean meal is often unprofitable for producers because of their high cost.

Therefore research efforts had been geared towards the use of locally available feedstuffs such as agro-industrial by-products and farm wastes which are less expensive when compared to conventional feed stuffs so that they can be used as a substitute for conventional sources of protein such as fish meal and soya bean meal.

Shrimp waste meal is one of such unconventional protein source that has the potential of being an alternative source of protein in broiler rations, partially or totally replacing conventional protein sources like fish meal. Shrimp waste meal is the dried and milled waste of the shrimp industry which consists of heads, shells and appendages of shrimp (Ingweye *et al*., 2008). During shrimp processing, the peeling step generates large amount of solid waste. Continuous production of shrimp waste with-out corresponding technology of utilizing the waste has resulted in waste collection, disposal and pollution problem. Shrimp waste can be potentially channeled as a substitute for fish meal in poultry diets.

Shrimp waste is the concentrated product containing proteins with characters of low cost, moisture, deodorized, high digestibility, easy storage that does not require refrigeration and have long shelf life. Shrimp waste contains high CP content and reasonably good balance of essential amino acids (Ngoan *et al*., 2000). It is particularly rich in lysine which makes it an ideal supplement for cereals (Fanimo *et al*., 1996). It is palatable and has a pleasant aroma.

Shrimp waste contains 7.87% of water, 26.89% of crude fiber, 24.03% of crude protein, 5.14% of crude fat, 25.60% of ash, 16.69% of calcium and 930 kcal ME/kg (Mahata *et al*., 2008). Shrimp waste apart from supplying good quality proteins and vitamins A and D, also contains several dietary minerals such as ca, Fe etc.

Shrimp meal (SM) can be used as a protein source in poultry diets (Fanimo *et al*., 1996; Rosenfeld *et al.,* 1997; Gernat, 2001; Oduguwa *et al.,* 2004; Khempaka *et al.,*2006a,) or as a source of prebiotic (Khempaka *et al.,* 2011, Zhou *et al*., 2009) with varying results.

The potentials of shrimp waste meal (SWM) as an alternative protein source in monogastric feeding is not in doubt (Talabi, 1988; Fox *et al.*, 1994). However, the extent of its usefulness and levels of utilization by monogastric livestock is yet to be resolved. To date limited works are carried out in this aspect with variable results.

In view of the above the present study is aimed to evaluate the performance of broilers fed diets containing shrimp waste meal and amino acids with the following objectives.

1) To study the chemical composition of shrimp waste meal.
2) To study the effect of shrimp waste meal and amino acids inclusion on growth performance of broilers.
3) To study the effect of shrimp waste meal and amino acids inclusion on nutrient digestibility.
4) To study the effect of shrimp waste meal and amino acids inclusion on gut pathogen population.
5) To study the effect of shrimp waste meal and amino acids inclusion on serological parameters.

6) To study the effect of shrimp waste meal and amino acids inclusion on hematological parameters (RBC, WBC, and LYMPHOCYTE %)

7) To study the effect of shrimp waste meal and amino acids inclusion on carcass characteristics of broilers.

CHAPTER - II

REVIEW OF LITERATURE

The research work carried by various research workers regarding performance of broilers fed diets containing shrimp waste meal at various levels in terms of body weight gain, feed intake, feed efficiency, digestibility of nutrients, *E.coli* and *Salmonella* count of the cecal contents, serological parameters, hematological parameters and carcass traits are presented in this chapter under following sub heads.

1. **Chemical composition of shrimp waste meal**
2. **Growth performance**
3. **Digestibility of nutrients**
4. **Gut pathogen population (*E.coli and Salmonella*)**
5. **Serological parameters**
6. **Hematological parameters**
7. **Carcass traits of broilers**

2.1. Chemical composition of shrimp waste meal

Shrimp waste meal is a byproduct from shrimp processing industry and has a high nutritive value (Ngo and le, 2003). Shrimp waste is essentially composed of heads, shells, appendages and tails (Fanimo *et al.,*2000). The shrimp processing industry has been rapidly growing with significant increase in cultured shrimp production in the South-East Asian region. During shrimp processing the peeling step generates large amount of solid waste, because head and peel represent 40% of the shrimp weight (Gildberg and Stenberg, 2001). The shrimp processing waste generated in India is around 1.25 to 1.50 lakh tons per annum. The major components of the shrimp waste (DM basis) are protein (35-50%), chitin (15-25%), minerals (10-15%) and carotenoids (Ramyadevi *et al*., 2012).

Shrimp waste is an excellent source of protein (50-60% on DMB), very low in fat and calories. Shrimp waste apart from supplying good quality protein and vit. A & D, it also contains several dietary minerals such as Ca, Fe etc., which are beneficial to animals (Ravi chandran *et al.,* 2009). SWM is particularly rich in lysine, which makes it an ideal supplement for cereals (Fanimo *et al.,* 1996).

The chemical composition of shrimp waste meal as reported by various authors is presented in Table 1. CP value of SWM ranged from 20% (Okonkwo *et al.,* 2012) to 50.89% (Rosenfeld *et al.*, 1997), whereas Total ash ranged from 15.64% (Rosenfeld *et al.,* 1997) to 26.73% (Khempaka *et al.*, 2006), crude fibre ranged from 3.6% (Fanimo *et al.,* 1998) to 29.75% (Khempaka *et al.*, 2006) and crude fat from 0.94% (Khempaka *et al.,* 2006) to 10.28% (Khempaka *et al.,* 2011). This variation among studies may be due to the difference in shrimp species (Ngoan *et al.,* 2000; Heu *et al.,* 2003), source of shrimp waste meal (head/shell) (Meyers, 1986) and/or processing method, as these can affect the nutritional values of SWM.

Table: 1 Chemical composition (%) of shrimp waste meal as reported by different authors

CP	EE	TA	CF	Ca	P	ME (kcal/kg)	Reference
20.00	7.44	24.50	8.46	NA	NA	NA	Okonkwo *et al.*, 2012
31.58	9.49	19.67	19.82	NA	NA	4023 (GE)	Septinova *et al.*, 2012
36.69	10.28	21.77	19.49	4.92	1.20	1515	Khempaka *et al.*, 2011
48.30	5.75	NA	12.90	3.50	1.00	1870	Aktar *et al.*, 2011
32.50	9.80	26.60	8.70	NA	NA	NA	Ravi chandran *et al.,2009*
24.03	5.14	25.60	26.89	16.69	0.85	938	Mahata *et al.*, 2008
48.30	6.30	17.55	13.30	NA	NA	NA	Ingweye *et al.*, 2008
39.32	0.94	26.73	29.75	6.05	0.97	1,350	Khempaka *et al.*, 2006a
53.47	3.42	16.80	1.18	0.74	0.31	1312	Ojewola *et al.*, 2006
40.20	4.80	16.20	10.90	NA	NA	NA	Odugwa *et al.*, 2004
46.30	9.04	17.04	4.30	7.00	3.03	2500	Fanimo and Oduguwa, 1999
43.71	8.64	17.04	3.60	10.21	0.48	NA	Fanimo *et al.*, 1998
50.89	6.31	15.64	8.92	5.21	1.47	2397	Rosenfeld *et al.*, 1997

NA- Not available

According to NRC (1994) the calcium content of the shrimp waste is 16-50 times more than calcium in fish meal. Mahata *et al.,(*2008) found the calcium in shrimp waste hydrolysate as 16.35% and phosphorous as 0.83%.The calcium and phosphorous have an antagonistic relationship in the process of absorption in small intestine of the broilers. The high calcium content in broiler diet will reduce the absorption of phosphorous and high phosphorous in diet will lower the absorption of calcium. The calcium and phosphorous deposition will determine the formation of hydroxyapatite for bone compactness during the mineralization process.

Bronner (1987) stated that the contents of the poultry intestine is almost acidic than alkaline and Kheiri and Rahmani (2006) found that Ca might increase the intestinal pH and consequently affect the digestion and absorption of nutrients. High levels of Ca in diet changes the intestinal pH of broiler from acidic to alkaline, this is a possible cause of lower protein digestion and absorption. Scott *et al.* (1982) also stated that protein plays an important role for broiler growth and protein deficiency will decrease weight gain.

Properly collected, preserved and processed good quality SWM with reduced amount of bacterial activity is essential, which otherwise can produce a dicarboxylic reaction turning amino acids from animal protein into biogenic amines, resulting in a toxic effect with the possibility of reducing performance and livability in birds (Dale,1994).

The exo skeleton of the shrimp is mainly composed of chitin. Subasinghe,(1999) reported that the head, shell and hull of shrimp waste products contained 11, 27 and 24% chitin, respectively. Chitin is a linear polymer of N-acetyl-D-glycocyamine unit linked with β (1, 4) glycosidic bonds (Minoru *et al.,* 2002) and chitinase is the enzyme that catalyzes the hydrolysis of chitin to its simple monomer of N-acetyl-D-glycocyamine (Park *et al.,* 1997). Chitin physically blocks the access of digestive enzyme to protein and lipid, thus affecting the utilization of these nutrients (Castro *et al.,* 1989). About 10% of the crude protein in whole shrimp meal originates from chitin while up to 50% of the nitrogen in scale meal, originates from chitin (Gohl, 1975).

The major concern with the use of shrimp waste meal is its chitin content which is considered to have low digestibility when fed to animal (Austin *et al.,* 1981). Some species of birds produce chitinase in the proventriculus. In case of the chicken, amounts of chitinase produced are low (Jeuniaux and Cornelius, 1978). Even in species that produce useful levels of chitinase the energy value of chitin is very low, due to poor absorption (Jeuniaux and Cornelius, 1978; Karasov, 1990). Low levels of chitin (0.5%) in broiler diets may improve growth performance.

Fanimo *et al.* (2000) assessed protein quality of SWM in a balance experiment with rats and results indicated that SWM is inferior to that of fish meal but that supplemental methionine and lysine in SWM diets improved the quality of protein. Ngoan *et al.* (2000) indicated that the amino acid composition of shrimp waste was fairly balanced, but the low methionine content can limit its value for mono-gastric animals. Other factors such as high chitin and calcium contents, could limit the amount of shrimp waste in mono-gastric diets. Chitin has low digestibility when fed to animals.

Chen *et al*. (2002) reported that degradation of chitin in SWM may give rise to physiological effects including anti-microbial and immune enhancing activity. Chitin digestibility in broilers has reported to be as low as about 20% (Khempaka *et al.*, 2006 b), although chitinolytic activity occurred in mucosa of the proventriculus in broilers (Koh and Iwamae, 2013).

Mustanur and Katsuki, (2014) reported that in Comparison with hulls, shrimp heads were significantly rich in crude protein (CP) and ether extract (EE) and poor in crude fibre (CF), Total ash (TA) and chitin. Overall, *in vitro* dry matter (DM) and CP digestibilities were significantly greater in heads than in hulls, which is reasonable because the level of chitin, non-digestible amino polysaccharides, were greater in hulls than heads in all species. Consequently, heads are considered to be more preferable than hulls as a source to generate a good nutritional quality SWM.

2.2 *IN VIVO* IMPACT OF SHRIMP WASTE MEAL ON BROILER PERFORMANCE

2.2.1 Effect of supplementation of shrimp waste meal on body weight gain

Damron *et al*. (1964) and Raab *et al*. (1971) incorporated shrimp waste meal at 9.1 and 6.8% in broiler diets and found no statistical difference in performance. Ilian *et al*. (1985) used shrimp meal, at levels above 10% and found no negative effect regarding broiler productive variables. Islam *et al.* (1994) reported that chickens receiving the diets containing 14.3% shrimp meal did not show negative effects on body weight gain.

Fanimo *et al.* (1996) reported non significant differences regarding body weight gain of broilers both at starter and finisher phase among treatment groups where fish meal contribution to dietary CP of broiler diets was replaced with SWM at 33 % level, however significant reduction of body weight gain was noticed at 66 and 100 % level of replacement. Fanimo suggested that supplementation of shrimp waste meal diets with synthetic methionine and lysine improve the utilization of shrimp waste protein, because the protein quality of shrimp waste meal is inferior to that of fish meal.

Razdan *et al.*, (1997) observed that decreased feed intake and body weight gain when broiler chicks fed with high dietary chitosan (30g/kg) concentration in diets might be induced by the high viscosity and slow motility of the chitosan in the gastrointestinal lumen, stimulating the satiety centre of the brain.

Aron (1985) reported that decreased weight gain was noticed in the birds fed with diets in which FM protein was completely replaced with SWM protein due to the high fibre and chitin in SWM which may have caused an imbalance in the levels of amino acids in the diet and the sparing effect of one amino acid upon the other.

Arellano *et al.* (1997) carried out studies with shrimp meal, by including it in broiler rations at 3, 6 and 9% and found no statistical differences ($P>0.01$) in weight gain per bird. Oduguwa *et al.* (1999) reported that there was significant reduction in the growth performance of weaner pig when fish meal was totally replaced by shrimp meal in diets. Ngoan et *al.*, (2000) also reported that complete replacement of the fish meal protein with shrimp meal protein reduced the performance of fattening pig.

Fanimo *et al.* (2004) conducted a growth trial on pigs. He reported that the pigs receiving Diet-1(control) containing (corn soya bean diet with fish meal) had highest live weight gain than the pigs receiving Diet -2 (Fish meal in control was replaced by shrimp meal). He concluded that feeding shrimp meal at a higher level to replace fish meal will have detrimental effects on the pig performance. The use of SWM protein instead of FM led to reduction in weight gain of pigs as the availability of amino acids in SWM is less (Gohl, 1975). He reported that about 10% of the crude protein in whole shrimp meal originates from chitin while up to 50% of the nitrogen in scale meal originates from chitin which is nearly indigestible. Hector and Lourdes, (2005) reported that body weights of broilers decreased significantly as percentage of shrimp meal increased beyond 6 % in the diet.

Okoye *et al.* (2005) concluded that dietary treatments (0%, 10%, 20% and 30% SWM) had significant effect ($P<0.05$) on body weight gain at the starter phase but not at finisher phase. At the starter phase birds fed 0 % and 10 % SWM diets had statistically comparable weight gain while those fed 20 %, 30 % SWM diets had depressed weight gain at starter phase. At finisher phase, all diets were comparable regarding body weight gain. The decline in performance of birds fed 20 and 30% SWM diets was observed especially at the starter phase. Increased inclusion level of SWM in broiler diets leads to an increase in the total chitin content in the diet resulting in low digestibility of nutrients and absorption in the gastro intestinal tract of broilers. The comparable performance observed at the finisher phase could be as a result of maturity and or age. This is because, as the birds increase in age the gastro intestinal tract and absorption capacity become more efficient in carrying out digestive processes. An indication that older birds were better able to take up chitin than the younger birds.

Ojewola *and* Annah, (2006) reported that non significant differences (P>0.05) were noticed regarding body weight gain among different dietary treatments with 6 % Danish fish meal, 6 % Cray fish dust meal, 6 % shrimp waste meal, 3% Cray fish dust meal+3% shrimp waste meal, 3 % Danish fish meal + 3 % Cray fish dust meal, 3 % Danish fish meal+ 3 % shrimp waste meal.

Khempaka *et al*. (2006) conducted feeding trials with broilers by incorporating SWM at 0%, 4%, 8%, 12% and 16%. There was no significant difference in body weight gains up to 8% inclusion of SWM, but there was significant decrease in body weight gain at 12% and 16% inclusion of SWM in comparison with control and 4% inclusion of SWM. They concluded that decreased body weight gain might have resulted from decreased feed intake and DM digestibility. The SWM used in the study was rich in fiber, ash and poor in CP. It is rich in chitin which might have decreased digestibility and high levels of chitin and or calcium in SWM are possible factors involved in decreased performance.

Ingweye *et al.* (2008) stated that no significant difference was observed regarding body weight gain in broilers in control (FM replaced with 0% Fish waste meal-FWM) and 25% FWM replaced with SWM but 100% replacement level had least weight gain which did not differ significantly with 50% and 75% replacement levels. The initial slow growth rate with increase in the quantity of SWM in the diet could be inability of birds to handle effectively the highly chitinous diets at tender age.

Mahata *et al.* (2008) reported that statistical analysis showed significant difference among dietary treatments (0, 4, 8 and 12% SWH) regarding the effects of shrimp waste hydrolysate towards weight gain. Beyond 8% level i.e. birds given diets containing 12% SWH had significantly decreased body weight gain when compared to birds fed other diets .

Iyamu *et al.* (2009) conducted a growth trial in broilers. He incorporated shrimp meal at 0, 25, 50, 75 and 100% levels by replacing fish meal and concluded that there was no significant difference between treatment mean for average weight gain. He suggested that shrimp waste meal could replace fish meal protein in diets of broilers.

Khempaka *et al.* (2011) concluded that inclusion of shrimp waste meal up to 15% did not result in any negative effect on body weight gain. Interestingly the addition of 5% SWM resulted in greater difference in body weight gain when compared to those birds fed control diet, but no significant difference (P>0.05) was observed between these two groups fed with 5% and 15% inclusion of SWM.

Aktar *et al.* (2011) reported that final live weight was highest in diet 4 (Control fish meal -FM diet replaced with 6 % meat waste-MW and 6 % shrimp waste-SW), Intermediate in diet 2 (Control FM diet replaced with 12 % MW) and diet 3 (Control FM diet replaced with 12 % SW) and lowest in diet 1 (control diet with 12 % FM).

Okonkwo *et al.* (2012) reported that there were no significant differences regarding body weight gain among treatment means even though the values showed a normal variation, and the values were 290.42 (T_1-0% SWM), 328.34 (T_2-5% SWM), 274.96 (T_3-10% SWM) and 303.75 (T_4-15% SWM) when birds are fed with diets containing SWM.

Chelladurai *et al.* (2014) conducted experiment on Koi carp fishes. He replaced fish meal with shrimp meal at 0, 25, 50, 75 and 100% levels. The fishes fed 25 and 50% shrimp waste had better weight gain than those fed with 75% and 100% shrimp waste meal but there was no significant difference in weight gain among the fishes fed diets with 75% and 100% shrimp waste replacement. He concluded that shrimp waste meal can effectively replace fish meal up to 50% in diet of koi carp fishes without affecting their growth rate.

2.2.2 Effect of supplementation of shrimp waste meal on feed intake

Fanimo *et al.*, (1996) reported that non significant differences were observed regarding feed intake among treatment groups where fish meal contribution to dietary CP of broiler diets was replaced with SWM at 0, 33, 66 and 100 % of graded levels. Arellano *et al*. (1997) found that inclusion of shrimp meal at 3, 6, 9% levels in diets of broilers produced non significant effects on feed consumption.

Gernat, (2001) reported a significant (P<0.01) increase in feed consumption with both 40 and 80% SWM compared to the 0% treatment. Experimental diets include -five different levels of SWM in the diet replacing 0, 20, 40, 60, or 80% of the SBM. These results might be attributed to the high levels of chitin found in SWM. Because chitin reduces dietary energy, the layers fed diets with higher levels of SWM increased feed consumption to maintain their energy needs.

Fanimo *et al.* (2004) reported that feed consumption (1.45 ± 0.02 kg) of grower pigs was similar when fed with the experimental diets containing, diet -1(corn-soya bean diet with fish meal (control) and diet -2 FM in control was replaced by shrimp meal). Oduguwa *et al*. (2004) concluded that feed consumption values at the starter and finisher phases revealed that birds fed the control diet (FM/SBM) consumed more than those on the other diets (SBM/SWM and FM/SWM) and the difference in the values between treatment groups (diet 2 and diet 3) were non significant (P>0.05).

Hector and Lourdes, (2005) reported non significant differences regarding feed intake among treatments groups (3%, 6% and 9% SWM). Okoye *et al*., (2005) concluded that dietary treatments (0%, 10%, 20% and

30% SWM) had significant effect on feed intake. At starter phase significantly highest feed intake was noticed in birds fed control diet and significantly lowest in birds fed diet with 30 % SWM. At finisher phase, feed intake was found to be significantly highest in birds fed diet containing 20 % SWM.

Khempaka *et al.* (2006) concluded that there was no significant difference in feed intake up to 8 % inclusion of SWM, but there was significant decrease in feed intake at 12 % and 16 % inclusion of SWM in comparison with control and 4 % inclusion of SWM. With increase in SWM in the diet, Ca level in diet was increased. It was reported that high levels (1.3-1.5 %) of calcium decreased feed intake in chickens (Smith and Taylor, 1961, Watkins *et al.*, 1989), in addition high levels of sodium in SWM has been reported to decrease feed intake (Damron and Kelly, 1987; Balog and Millar, 1989)

Ingweye *et al.* (2008) stated that no significant difference was observed regarding feed intake among dietary treatment groups. Replacement of fish meal with shrimp waste meal at 0 (T_1), 25 (T_2), 50 (T_3), 75 (T_4) and 100 % levels (T_5) showed that the feed intake ranged from 296.15±36.55 to 328.85±41.21 g/bird, 947.49 ±76.98 to 1048.13±69.79 g/bird and 621.82±71.33 to 688.49±57.08 g/bird for the starter, finisher and combined phases, respectively.

Mahata *et al.* (2008) reported that statistical analysis showed non significant difference with regard to feed consumption among treatment groups (0, 4, 8 and 12%). Iyamu *et al.* (2009) carried out studies in broilers where shrimp meal was incorporated at 0, 25, 50, 75, 100% of graded levels by replacing fish meal in the experimental diets. No statistical difference was found ($P \geq 0.05$) regarding feed consumption among treatment groups.

Aktar *et al.* (2011) reported that feed consumption of broilers at 28 days of age was similar in all experimental diets ($P>0.05$). At 42, 56 days of age highest feed intake was observed in broilers receiving diet 1 (control diet with 12 % Fish meal) and lowest on diet 4 (Fish meal of the control diet replaced with 6% Meat waste and 6% Shrimp waste) and intermediate in diet 2 (Fish meal of the control diet replaced with 12 % Meat waste) and diet 3 (Fish meal of the control diet replaced with 12 % Shrimp waste).

Khempaka *et al.* (2011) reported non significant differences with regard to feed intake among treatment groups (control, 5%, 10%, 15%, 20 % Shrimp waste meal diets). Okonkwo *et al.* (2012) showed that non significant differences were observed regarding feed intake among treatment means inspite of the variation in values and the feed intake (g) was 1614.57 (T_1-0% SWM), 1549.00 (T_2-5% SWM), 1525.71 (T_3-10% SWM) and 1541.29 (T_4-15% SWM).

2.2.3 Effect of supplementation of shrimp waste meal on feed conversion ratio (FCR)

Fanimo *et al*. (1996) reported non significant differences with regard to FCR of broilers at finisher phase among treatment groups where fish meal contribution to dietary CP of broiler diets was replaced with SWM at 33% and

66% level, however significant difference (poor efficiency) was noticed at 100% level of replacement.

Arellano *et al.* (1997) conducted a feeding trial in broilers and he reported that the feed efficiency was poorer for the diets containing shrimp meal at 40% and 80% level. The feed efficiency was poorer because of the increase in feed consumption in those dietary treatments.

Fanimo *et al.* (2004) reported that there was a significant difference ($P < 0.05$) in the feed conversion ratio in growing pigs fed with the experimental diets (Diet – 1(control) corn-soy bean diet with fish meal , Diet – 2 Fish meal in control was replaced with shrimp meal). FCR was reduced with the diet containing shrimp meal.

Experimental reports of Oduguwa *et al.*, (2004) revealed that birds fed with control diet (FM/SB) had best FCR than diet 2 (SBM/SWM) and least with diet 3 (FM/SWM) both during starter and finisher phases. Hector and Lourdes, (2005) reported that FCR was similar for all treatments (control, 3%, 6% and 9% SWM) at starter phase however at finisher phase birds fed control diet (2.44) and birds fed 3% SWM (2.71) had significantly lower FCR than those fed 9% SWM (4.50), while FCR of birds fed 6% SWM was comparable with other treatments (control, 3% and 9 %).

Okoye *et al.*, (2005) concluded that dietary treatments at 0%, 10%, 20% and 30% SWM had significant effect on feed to gain ratio at the starter phase but not at finisher phase. At the starter phase birds fed with 0% and 10% SWM diets had statistically comparable feed to gain ratio while those fed 20%, 30% SWM diets had significantly ($P<0.05$) higher feed to gain ratio. At finisher phase, all diets were comparable regarding feed to gain ratio.

Ojewola and Annah, (2006) reported non significant differences ($P>0.05$) regarding feed conversion ratio among different dietary treatments (6% Danish fish meal; 6% cray fish dust meal; 6% shrimp waste meal; 3% Cray fish dust meal + 3% shrimp waste meal; 3% Danish fish meal + 3% Cray fish dust meal; 3% Danish fish meal + 3% Shrimp waste meal).

Khempaka *et al.*, (2006) concluded that there was no significant difference in feed efficiency up to 8% inclusion of SWM, but there was significant ($P<0.05$) decrease in feed efficiency at 12% and 16% inclusion of SWM in comparison with control and 4 % inclusion of SWM.

Ingweye *et al.* (2008) reported that FCR was best at 0% level of replacement and higher values were recorded for 100% replacement level which did not differ significantly ($P>0.05$) with 50 and 75% replacement level and thus concluded that 25% level of replacement of FWM with SWM was optimum for broiler performance. The feed conversion ratios were best ($P<0.05$) at the 0% replacement level for all the phases i.e., 1.45 ± 0.12, 1.90 ± 0.13 and 1.67 ± 0.12 for the starter, finisher and combined phases respectively. These were not different ($P>0.05$) from the control in all the phases. The worst values ($P<0.05$) were 3.50 ± 0.22, 3.42 ± 0.25 and 3.46 ± 0.31 recorded for the starter, finisher and combined phases respectively, at 100%

replacement level. Feed conversion ratio was inversely related to the feed intake. As the level of shrimp waste in the diet increased, feed conversion ratio increased. This could be as a reflection of increasing feed intake and decreasing weight gain.

Mahata *et al*. (2008) reported that the statistical analysis showed significant difference among dietary treatments (0, 4, 8 and 12% Shrimp waste hydrolysate) regarding FCR. The FCR beyond 8% level of inclusion i.e birds fed diet with 12 % SWH had significantly higher FCR when compared to birds fed other experimental diets. Khempaka *et al*. (2011) reported that FCR did not change significantly when SWM was at or below 15%. Interestingly the addition of 5% SWM resulted in greater difference in FCR (1.88) when compared to those birds fed control diet (1.95), but no significant difference ($P>0.05$) was observed between these two groups (control and 5% SWM).

Iyamu *et al.* (2009) reported that there was no statistical difference ($P \geq 0.05$) among dietary treatments (0, 25, 50, 75, 100%) regarding FCR. Aktar *et al*. (2011) reported that feed conversion of broilers at 28 days of age did not differ significantly ($P>0.05$) among experimental diets. But at 56 days of age highest feed conversion was observed with diet 4 (FM of Control diet replaced with 6% MW and 6% SW) and lowest with diet 1 (control diet with 12% FM) ($P<0.01$).

Okonkwo *et al*. (2012) showed that feed conversion ratio did not differ significantly among dietary treatments (T_1-0% SWM), (T_2-5% SWM), (T_3-10%

SWM) and (T_4-15% SWM) but numerically lower values were observed in SWM supplemented groups when compared to control.

2.3 Effect of supplementation of shrimp waste meal on nutrient digestibility

The enhanced ileal digestibility of nutrients in the broilers fed chitin oligosaccharide containing diets might be due to reduced number of pathogenic bacteria like *Escherichia coli, Salmonella typhimurium* (Choi *et al*., 1994; LeM- ieux *et al*., 2003; Wang *et al*., 2003., Boyle *et al*., 2007; McNulty *et al*., 2007) and increase in the number of the beneficial bacteria like *Lactobacilli* (Oli *et al*., 1998) in the intestine. Such changes in the intestinal bacterial population resulted in a decrease in the incidence of diarrhoea (Oli *et al.,* 1998) and increase in immune function (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003).

chito oligosaccharides may stimulate the secretion of digestive enzymes from the stomach, pancreas and intestinal mucosa (Hou and Gao, 2001). This effect is expected to reduce local inflammation in the intestinal mucosa, facilitate the breakdown of complex molecules into simpler ones and enhance the integrity of enterocytes, thereby promoting the digestion and absorption of nutrients (Wu, 1998). Through an increase in the digestion and absorption of nutrients, dietary supplementation with COS reduces the excretion of fecal nitrogen and phosphorus from animals, thereby minimizing the major sources of environmental pollution.

Dietary supplementation of 100 mg/kg of COS to broilers was as effective as well-documented antibiotics (6 mg/kg of flavomycin) in enhancing the ileal digestibility of nutrients and average daily gain, compared with the broilers fed the basal diet. Thus, compared with feeding an antibiotic, dietary COS supplementation to poultry and other livestock species offers three unique advantages: 1) preventing drug resistance in animals and humans; 2) improving the health of the small intestine; and 3) increasing the ileal digestibility of dietary phosphorus (Huang *et al*., 2005).

Fanimo *et al.* (2004) reported that replacing fish meal with shrimp meal in the diets of growing pigs reduced the digestibility of DM, CP, CF and Ash by 7.1, 9.44, 11.73 and 14.81% respectively. Chitin physically blocks the access of digestive enzymes to lipids and protein thus effecting the utilization of these nutrients (Castro *et al.,* 1989, Karasov, 1990).

Khempaka *et al.* (2006) concluded that there were no significant differences regarding DM digestibility and nitrogen retention up to 8 % inclusion of SWM, but there was significant reduction at 12% and 16% inclusion of SWM in comparison with control and 4% inclusion of SWM.

Ojewola and Annah, (2006) reported that non significant differences were observed regarding fat, ash, crude fiber digestibility and nitrogen retention among treatment groups (6% Danish fish meal; 6% Cray fish dust meal; 6% shrimp waste meal; 3% Cray fish dust meal + 3% Shrimp waste meal; 3% Danish fish meal + 3% Cray fish dust meal; 3% Danish fish meal + 3% Shrimp waste meal).

Mahata *et al.* (2008) reported that statistical analysis showed significant difference among dietary treatments regarding effects of shrimp waste hydrolysate toward nitrogen retention beyond 8% level of inclusion i.e birds fed diet with 12% SWH had significantly lower nitrogen retention values when compared to birds fed other diets. Khempaka *et al.* (2011) reported that DM, OM and Ash digestibility values and nitrogen retention did not change significantly when SWM was at or below 15 %.

Khambulai et al. (2008 and 2009) reported that supplementation of low dietary chitosan (0.6g/kg) concentration until 7 weeks of age in marshall chunky broiler chicks had increased feed intake and body weight gain due to better absorption of nutrients.

2.4 Effect of supplementation of Shrimp waste meal on microbial count

The use of shrimp waste meal as a protein source in poultry diets have a beneficial effect, it can potentially alter the microbial ecology of the intestine. Acidic digestive fluid in the proventriculus and gizzard may degrade the shrimp shell to release chitin. In the neutral pH of the small intestine, chitin would gradually precipitate and move into the large intestine (mainly the caeca), where microbes may release enzymes to hydrolyze chitin.

The degradation products of chitin can inhibit the growth of harmful bacteria (Chen *et al*., 2002) and enhance the growth of *Bacteroides* spp. in the ceca of rats (Chen and Chen, 1999). It is well known that the microbial community and its activity play important role in the intestinal, physiological, immunological and protective functions of the poultry intestinal tract and can be influenced by diet composition.

Salmonella spp., pathogenic *E. coli,* considered to be food borne pathogens and they are commonly found in the gastrointestinal tract of poultry. Izat *et al*. (1991) reported that poultry products are frequently contaminated with various serotypes of *Salmonella* and with *E. coli* strain O157:H7. Salmonellosis in humans is most frequently associated with the consumption of contaminated fresh and processed poultry products (Lynch *et al*., 2006; Foley *et al.*, 2011). According to Foley *et al.* (2011) *Salmonella Typhimurium* continues to be among the most common serovars isolated from poultry and a common cause of human salmonellosis. Furthermore, public concern associated with antibacterial strains is challenging the poultry industry to find alternative means of control and consequently, continuous studies on alternative methods to control food borne pathogens are necessary (Boyle *et al.*, 2007; McNulty *et al.*, 2007 and Menconi *et al.*, 2014). Feeding SWM has shown to enhance the growth of *Lactobacillus* and to inhibit the growth of *E. coli* and *Salmonella* in the intestine.

Khempaka *et al*. (2006) reported that approximately 20% of chitin can be digested in the gastrointestinal tract of broilers. Chen *et al*. (2002) reported that degradation of chitin in SWM may give rise to physiological effects including anti-microbial and immune enhancing activity.

Li *et al.* (2007) reported that the concentration of *E.coli* in the caecum was significantly decreased by dietary supplementation of 100 mg/kg of chito-oligosaccharide in comparison with the control birds. Khempaka *et al*. (2011) reported that feeding broilers with SWM resulted in increased population of *lacto bacillus* and decreased intestinal *Escherichia coli* and cecal *Salmonella.* Menconi *et al.* (2014) reported that *in vivo* reduction in cecal *Salmonella Typhimurium* (ST) may decrease the overall pathogen load in birds, making them less likely to spread the infection further. They reported that the addition of 0.2% chitosan in broiler diet proved to be an effective alternative tool to reduce colonization of ST in broiler chickens and significantly reduced ST counts in crop and cecal content leading to reduced carcass ST contamination as well as decreasing the amount of ST shed to the environment.

2.5 Effect of supplementation of shrimp waste meal on serum constituents (Protein, Albumin, Globulin, Cholesterol, Glucose)

Kobayashi and Itoh, (1991) reported that dietary chitosan inhibited the increases in plasma triglyceride concentration and abdominal fat weight in laying type chicks fed high-fat diets.

Olayemi, (2001) reported that non significant differences were observed regarding serum metabolites among dietary treatments T_1 (soya bean & fish meal), T_2 (FM was replaced with SWM), T_3 (SBM was replaced with SWM). Li *et al*., (2007) reported that higher serum total protein content was observed when broilers were supplemented with chito-oligosaccharide at 100 mg/kg level than other treatment birds.

The feeding of chito-oligosaccharides to pigs also favorably alterered whole-body lipid metabolism (Jabbal *et al*., 1998; Wang, 1998; Tang *et al*., 2005) and increased serum levels of total protein, growth hormone, and insulin-like growth factor-I as well as hepatic and m RNA levels for insulin-like growth factor- I (Tang *et al*., 2005).

Dietary supplementation of chito-oligosaccharides increased serum growth hormone and the insulin-like growth factor-I (IGF-I), mRNA level and enhanced protein synthesis in early-weaned pigs (Tang *et al.*, 2005, Li *et al.,* 2007).

Zhou *et al*. (2009) reported that chito oligosaccharide supplementation had no effect on the total protein in treatment and control groups. Abiodun Adeyeye *et al.* (2014) reported that the levels of serum cholesterol increased as the level of SWM substitution increased in turkey poults.

2.6 Effect of supplementation of shrimp waste meal on hematological parameters

Meng *et al*. (2010) reported an increase in the concentration of WBC when laying hens were fed chito-oligosaccharides at 0.4 % of diet. Chen *et al.* (2009) concluded that 5g/kg of chito-oligosaccharides supplementation added in the diet did not affect the concentration of WBC, RBC and lymphocyte.

Zhou *et al*. (2009) reported that the RBC counts were 18.2% greater in birds fed chito-oligosaccharides at 0.4 % of diet than in birds in the control group. However, chito-oligosaccharide supplementation had no effect on the white blood cells and lymphocytes.

2.7 Effect of supplementation of shrimp waste meal on broiler carcass traits

Fanimo *et al.* (1998) reported increase in the liver weight and decrease in plucked weight with increase in shrimp waste meal in the diet. However, no significant difference was reported regarding dressing percentage among treatment groups (dietary CP contributed by FM was replaced with SWM at 0, 33, 66 and 100 % graded levels).

Olayemi, (2001) reported non significant differences regarding gizzard, spleen, kidney and lung weight but heart & liver weight varied significantly among treatment groups (T_1-soya bean & fish meal, T_2-FM was replaced with SWM, T_3-SBM was replaced with SWM). Cunha *et al.* (2003b) reported that no significant differences were observed regarding breast, thigh and drumstick yield of broilers fed 0, 3, 6, 9 and 12 % Shrimp waste meal.

Agunbiade *et* al. (2004) reported non significant differences among treatment groups for liver, heart and gizzard weight in broilers fed cassava products diets supplemented with Shrimp waste meal. Hector and Lourdes, (2005) reported that final live body weight and plucked carcass weight were similar for control, 3% and 6% SWM treatments but significantly higher than

9% fed group. Dressed carcass weight of control and 3% SWM fed groups was significantly higher than 6% and 9% SWM diets.

Mahata *et al*. (2008) reported that statistical analysis showed non significant differences among dietary treatments (0, 4, 8 and 12% SWH) with regard to carcass percentage. The average percent of digestive organs: liver, proventriculus and gizzard were not significantly affected by shrimp waste supplementation.

Aktar *et al*. (2011) reported that dressed yield, total meat and drumstick meat were highest (P<0.01) on diet 1 (control diet with 12 % FM) and diet 4 (FM of control diet replaced with 6% MW and 6% SW) and intermediate in diet 2 (FM of control diet replaced with 12% MW) and lowest in diet 3 (FM of control diet replaced with 12% SW) and other meat yield characteristics were not influenced by diet (P>0.05).

Okonkwo *et al*. (2012) reported non significant (P>0.05) differences among treatment groups (T_1- 0% SWM, T_2 -5% SWM, T_3-10% SWM and T_4-15% SWM) for plucked weight, dressed weight, eviscerated weight, neck, wing, thigh/drumstick, breast, gizzard, liver and heart weights.

2.8 Effect of supplementation of shrimp waste meal on mortality

Jarquin *et al.* (1972) found higher mortality in birds fed on diet where shrimp by-product replaced fish meal of control diet. Islam *et al.* (1994) reported that survivability was similar in control fish meal diet and test diets in which 50% dietary fish meal was replaced with shrimp waste. However, survivability was reduced with diet in which 75% dietary fish meal was replaced by shrimp waste.

Fanimo *et al. (*1998) reported that mortality increased with level of SWM in the diet at the starter phase. This may be due to the inability of the chicks at this early age to cope up with the chitin level in SWM. Cases of leg problems were observed during the early stage of the birds but they later overcame it. These observed leg problems may be due to the high ash or mineral content (especially Ca:P ratio) of the SWM which may lead to mineral imbalance. Calcium carbonate is responsible for the scleratization of the exoskeleton and represents most of the mineral matter. Because of its high mineral level, SWM is usually used in combination with other protein supplements (Meyer and Rutledge, 1971).

Gernat, (2001) reported non significant differences among treatment groups with regard to mortality. Mortalities for all treatments (five different levels of SM in the diet replacing 0, 20, 40, 60, or 80% of the SBM) were less than 1%. Aktar *et al*. (2011) reported that survivability was not affected by different dietary treatments (control diet with 12% Fish meal, FM of control diet replaced with 12% meat waste, FM of control diet replaced with 12% Shrimp waste, FM of control diet replaced with 6% meat waste and 6% shrimp waste).

CHAPTER- III

MATERIALS AND METHODS

The research work was planned to evaluate the performance of broilers fed diets containing fish meal protein replaced by shrimp waste meal protein with and without synthetic amino acids supplementation. The biological experiment was carried out at the Department of Animal Nutrition and Department of I.L.FC, College of Veterinary Science, Tirupati. Using the facilities of Department of Poultry Science, CVSc, Tirupati. Lab analysis was carried out at Departments of Animal Nutrition, Veterinary Microbiology and State Level Animal Disease Diagnostic Laboratory, College of Veterinary Science, Tirupati.

The experimental design and analytical methods followed during study period are presented in this chapter.

3.1 PROCUREMENT OF FEED INGREDIENTS

Feed ingredients like maize, soybean meal, fish meal and de-oiled rice bran (DORB) for preparation of experimental diets were procured from the local market. Shrimp waste meal was procured from Om Sai Aqua Industry, Venkateswarapuram, Nellore district.

3.2 CHEMICAL ANALYSIS

3.2.1 Proximate analysis of feed ingredients

Representative samples of feed ingredients were analyzed for their proximate composition (AOAC, 2005). The ME values for all feed ingredients except fish meal was estimated using equation suggested by NRC poultry (1994), while for fish meal the ME value was derived as per equation suggested by Leeson and Summers (2008).

Maize

ME_n(kcal/kg) = 36.21× CP+85.44×EE+37.26×NFE

DORB

ME_n (kcal/kg) = 46.7×DM-46.7×Ash-69.54×CP+42.94× EE-81.95×CF

SOYBEAN

ME_n(kcal/kg) = 37.5× CP+70.52×EE+14.9×NFE

Fish meal

ME_n (kcal/kg) = 3000(Deviation in % fat× 8600) ± (Deviation in % CP× 3900)

Where standard fat content is 2% and CP is 60%

3.2.2 Estimation of calcium and phosphorous

Representative samples of feed ingredients and broiler diets were analyzed for calcium and phosphorous as per the method of Talapatra *et al.*, (1940).

3.2.3 Experimental diets

Table 2: Experimental diets (Pre-starter)

Treatments	Contents of the experimental diets
T_1	Standard diet (control group)
T_2	Standard diet containing shrimp waste meal by replacing 20% of FISH MEAL protein of control diet
T_3	Standard diet containing shrimp waste meal by replacing 30% of FISH MEAL protein of control diet.
T_4	T_2+synthetic lysine, methionine.
T_5	T_3+synthetic lysine, methionine.

Table 2.1: Experimental diets (starter and finisher)

Treatments	Contents of the experimental diets
T_1	Standard diet (control group)
T_2	Standard diet containing shrimp waste meal by replacing 50% of FISH MEAL protein of control diet
T_3	Standard diet containing shrimp waste meal by replacing 100% of FISH MEAL protein of control diet.
T_4	T_2+synthetic lysine, methionine.
T_5	T_3+synthetic lysine, methionine.

Experimental diets (Table 2,2.1) were formulated for pre-starter (0-14 days), starter (15-28 days) and finisher (29-42 days) phases and their ingredient composition along with calculated values for important nutrients are presented in tables 3,4 and 5 respectively. All the five diets during each phase were iso-nitrogenous and iso-caloric and other ingredients like palm oil, L-Lysine and DL-Methionine were also used to meet the dietary concentration of energy, protein, lysine and methionine as per ICAR 2013 specifications for broiler diets.

As per the earlier findings of Fanimo *et al.* (1996) who reported that the mortality increased with the due increase in levels of SWM inclusion in broiler diets at the chick stage, which can be attributed to the presence of high levels of chitin in diets and it was not tolerated by young birds, cases of leg problems ,decreased performance were reported during pre-starter phase. At starter and finisher phases as the birds increase in age the gastro intestinal tract and absorption capacity become more efficient in carrying out digestive processes indicating that the older birds were more efficient to take up chitin than the younger birds.

Hence, in this experimental study shrimp waste meal was added by replacing 20% and 30% of FISH MEAL protein of control diet in pre-starter phase and in starter and finisher phases SWM was added at 50 and 100% levels to replace protein from FM of control diet.

Table 3: Ingredient composition (%) and chemical composition (%) of broiler pre-starter experimental diets

Ingredients	T_1	T_2	T_3	T_4	T_5
Maize	58	57.9	58	57.9	58
Soybean meal	20.55	20.4	20.3	20.34	20.21
Fish meal	10	8	7	8	7.0
Shrimp waste meal	0	2.18	3.27	2.18	3.27
De-oiled rice bran	5	5	4.83	5	4.83
Palm oil	3.7	3.77	3.85	3.77	3.85
Mineral mixture*	2	2.0	2.0	2.0	2.0
DL-methionine	0.2	0.2	0.2	0.21	0.21
L-lysine	0.55	0.55	0.55	0.6	0.63
Feed additives**	+	+	+	+	+
Total	100	100	100	100	100
Chemical composition					
CP	22.1	22.05	22.0	22.08	22.04
ME (kcal/kg)	3002	2998	2999	2997	3000
Lysine	1.18	1.15	1.13	1.20	1.19
Methionine	0.51	0.51	0.50	0.52	0.51
Ca	1.01	1.02	1.02	1.02	1.02
Available P	0.7	0.66	0.63	0.66	0.63
Cost (Rs/kg)	28.7	28.96	28.89	29.04	29.04

** Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm.*

*** All diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8mg; Vit-B_6, 16 mg Vit-B_{12}, 80µg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg ,Merivite®-AB_2D_3K@10g/100kg :(Each gram contains: Vit-A 82,500 IU, Vit-B_2 52mg, Vit-D_3 1200 IU, Vit-K 10mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3-5, Dinitro-O-Toluamide:25 percent W/W)*

Table 4: Ingredient composition (%) and chemical composition (%) of broiler starter experimental diets

Ingredients	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Maize	59	60.25	59.51	60.25	59.77
Soybean meal	19.3	19.5	19.8	19.39	19.3
Fish meal	10	5.0	0	5.0	0
Shrimp	0	5.45	10.89	5.45	10.89
De-oiled rice bran	5.0	3.0	2.5	3.0	2.5
Palm oil	4.1	4.2	4.7	4.2	4.7
Mineral mixture*	2.0	2.0	2.0	2.0	2.0
DL-methionine	0.17	0.17	0.17	0.18	0.2
L-lysine	0.43	0.43	0.43	0.53	0.64
Feed additives**	+	+	+	+	+
Total	100	100	100	100	100
Chemical composition					
CP	21.50	21.49	21.50	21.50	21.50
ME (kcal/kg)	3049	3049.3	3048	3047	3049.2
Lysine	1.09	0.99	0.99	1.01	1.01
Methionine	0.48	0.46	0.40	0.47	0.47
Ca	1.01	1.01	1.02	1.01	1.01
Available P	0.7	0.58	0.46	0.58	0.46
Cost (Rs/kg)	28.39	28.30	28.49	28.53	29.02

** Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm.*

*** All diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8mg; Vit-B_6, 16 mg Vit-B_{12}, 80µg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg ,Merivite®-AB_2D_3K@10g/100kg :(Each gram contains: Vit-A 82,500 IU, Vit-B_2 52mg, Vit-D_3 1200 IU, Vit-K 10mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3-5, Dinitro-O-Toluamide:25 percent W/W)*

Table 5: Ingredient composition (%) and chemical composition (%) of broiler finisher experimental diets

Ingredients	T_1	T_2	T_3	T_4	T_5
Maize	65	65	64.81	65	65.24
Soybean meal	14	14.2	14.7	14.06	14
Fish meal	10	5.0	0	5.0	0
Shrimp	0	5.45	10.89	5.45	10.89
De-oiled rice bran	4.8	3.65	2.5	3.65	2.5
Palm oil	3.8	4.3	4.7	4.3	4.7
Mineral mixture*	2.0	2.0	2.0	2.0	2.0
DL-methionine	0.1	0.1	0.1	0.11	0.13
L-lysine	0.3	0.3	0.3	0.43	0.54
Feed additives**	+	+	+	+	+
Total	100	100	100	100	100
Chemical composition					
CP	19.55	19.5	19.50	19.6	19.5
ME	3096	3102	3099	3099	3098
Lysine	0.92	0.83	0.74	0.93	0.92
Methionine	0.4	0.39	0.37	0.4	0.4
Ca	1.07	1.09	1.01	1.08	1.09
Available P	0.69	0.57	0.45	0.57	0.45
Cost (Rs/kg)	26.39	26.53	26.71	26.82	27.06

** Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm.*

*** All diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8mg; Vit-B_6, 16 mg Vit-B_{12}, 80µg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg ,Merivite®-AB_2D_3K@10g/100kg :(Each gram contains: Vit-A 82,500 IU, Vit-B_2 52mg, Vit-D_3 1200 IU, Vit-K 10mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3-5, Dinitro-O-Toluamide:25 percent W/W)*

3.3 Experimental design and birds

The experiment was carried out on three hundred and seventy five commercial day old Vencobb male broiler chicks obtained from a local hatchery for a period of 42 days in the Department of Animal Nutrition and Department of I.L.FC in collaboration with Department of Poultry Science, Tirupati. Permission has been taken from institute SPCA committee regarding housing management.

On arrival the broiler chicks were wing banded and weighed individually and distributed randomly on equal weight basis in to five treatments. Each treatment contained three replicates with twenty five birds per replicate.

3.4 Management and feeding

All the chicks were housed in well ventilated individual pens allotted to each replicate and reared in deep litter system in which paddy husk was used as litter material. All the pens were provided with uniform brooding facilities, linear feeders and trough waterers with sufficient feeder and water space per bird. Irrespective of the treatments, all the chicks were fed *ad lib.* for 3 times a day with respective broiler pre-starter diet from day old to 14 days, starter diet from 15 to 28 days and broiler finisher diet from 29 to 42 days of age. Fresh and clean drinking water was made available at all the time.

The chicks were vaccinated with HVT vaccine at day-old in hatchery, F_1 vaccine at 5^{th} day, IBD vaccine at 14^{th} day and Lasota vaccine at 22^{nd} day age of the birds. Except for feeding experimental diets, other managemental practices were uniform throughout the experimental period.

3.5 *In vivo* Studies

3.5.1 Body weight gain

The individual body weight of the birds was recorded at weekly interval up to 6 weeks of age. From this average weekly body weight gain per bird was calculated for each replicate in every treatment.

3.5.2 Feed intake and feed Efficiency

Weekly feed consumption was recorded replicate wise in every treatment and the feed efficiency was calculated accordingly.

3.5.3 Nutrient Digestibility

Digestibility trials were conducted during the starter and finisher phases of the biological trial. Two birds from each replicate, thus a total of six birds per treatment were kept separately in six metabolic cages. Birds in the cages were fed with the respective experimental diets consecutively for 3 days and the total feed offered was weighed and recorded for each cage. Similarly feces voided and feed left over in each cage was carefully collected, weighed and recorded. The representative samples of experimental diets offered and fecal samples from each cage were collected separately and analyzed for dry matter, crude protein, ether extract and crude fiber as per AOAC, (2005).

3.5.4 E. *coli and Salmonella* count of the intestinal contents

E.coli and *Salmonella* count of the intestinal contents were estimated at pre-starter, starter and finisher phases as per the procedure prescribed by American Public Health Association (1984). One gram of the intestinal contents pooled from the cecum was aseptically collected and mixed with 9 ml of sterile nutrient broth. Serial dilutions of each sample were prepared and the samples in duplicates were incubated at 37 ºC over a period of 24-48 hours. After incubation, one ml of the inoculum was transferred into petri plates containing EMB agar and BGA agar that were selective media for *E.coli and Salmonella* respectively. The inoculum was uniformly streaked throughout the plate by surface spread method. The plates meant for total counts were incubated at 37 ºC over a period of 24-48 hours. Those plates revealing visible colonies in the range of 30-300 were selected, counted and the counts were expressed as $\log_{10}$ (cfu/g) of sample.

3.5.5 Serum and hematological parameters

At the slaughter of the birds, blood samples were collected at the end of pre starter, starter and finisher phases from each bird for estimation of blood cell count (RBC, WBC, Lymphocyte %) in whole blood and serum was also separated to estimate serum metabolites. The separated serum was then made clear by centrifuging at 3000 RPM for 10 minutes and transferred to dry, clean epindorf tubes and stored in a refrigerator at (-20^{0}c) for estimation of serum parameters.

3.5.5.1Estimation of serum total protein

Serum total protein was estimated calorimetrically using diagnostic kit (M/s Span diagnostics limited) by Biuret method (Varley *et al.*, 1980)

3.5.5.2 Estimation of Serum albumin

Serum albumin was estimated calorimetrically by using diagnostic kit (M/s Robonic (India) PVT. LTD., as per the bromocresol green method (Doumas *et al.,* 1971)

3.5.5.3 Estimation of serum globulin

The globulin values were calculated by subtracting the values of serum albumin from the corresponding values of total protein.

3.5.5.4 *Estimation of serum glucose*

Serum glucose was estimated calorimetrically using diagnostic kit (M/s Robonic (India) PVT. LTD.,) by enzymatic GOD/POD method.

3.5.5.5 Estimation of Serum cholesterol

Serum cholesterol was estimated calorimetrically by using diagnostic kit (M/s Robonic (India) PVT. LTD.,) by enzymatic method of Allian, (1974) .

3.5.6 Carcass traits

At the end of the experiment, two birds from each replicate and thus a total of six birds per each treatment were randomly chosen, weighed and slaughtered. The liver, gizzard and heart were collected and weighed and the percentages were calculated on live weight basis.

3.5.7 Mortality

Mortality among the birds during entire experimental period was recorded and the causes there off were ascertained by detailed autopsy.

3.5.8 Cost economics

The relative economics of raising broilers up to six weeks of age with shrimp waste meal was calculated based on the present actual input costs, live weight and prevailing market price of broilers.

3.5.9 Statistical analysis

All the data obtained in this experiment were subjected to analysis of variance (Snedecor and Cochran, 1994).

CHAPTER - IV

RESULTS

The chemical composition of feed ingredients, experimental diets and performance of broilers fed diets containing shrimp waste meal and amino acids at various levels in terms of body weight gain, feed intake, feed efficiency, economics and mortality patterns are presented in this chapter. Further, effect of shrimp waste meal and amino acids on serological parameters, hematological parameters, and digestibility of nutrients in experimental diets, carcass traits and *E.coli*, *Salmonella* count of the cecal contents are also presented in this chapter.

4.1 NUTRIENT COMPOSITION

4.1.1 Chemical composition (%) of feed ingredients used in experimental diets

The nutrient content (%DM) of maize, soybean meal, fish meal and de-oiled rice bran used in the experimental diets are presented in the Table 6.

The percent DM, CP, EE, CF, TA and NFE of maize, soybean meal, fish meal and de-oiled rice bran were 90.2, 10.5, 1.5, 6.7, 6.3 and 75; 90.8, 45, 1.02, 9.46, 8.24 and 36.28; 91.17, 55, 2.5, 2.4, 20.5 and 19.6; 93.6, 13.0, 0.8, 16.8, 15.5 and 53.9, respectively. The per cent Ca, P content were 0.01, 0.13 (maize); 0.20, 0.3 (SBM); 6.5, 3.5 (FM) and 0.06, 0.8 (DORB), respectively. The percent lysine and methionine content of the feed ingredients were 0.2 and 0.2 (maize), 1.28 and 0.33(SBM), 3.50 and 1.19 (FM) and 0.51 and 0.29

(DORB), respectively. The Metabolisable energy (kcal/kg diet) estimated (Leeson and Summer, 2008) were 3300, 2300, 2200 and 1400 for maize, SBM, FM, and DORB, respectively. The proximate composition (%) of shrimp waste meal (table 6) revealed that it contained 94.17 (DM), 50.5 (CP), 8.2 (EE), 15.2 (CF), 19.1 (TA), 7.0 (NFE), 6.0 (Ca), 1.2 (P), and 1515 (kcal/kg diet) (ME).

4.1.2 Chemical composition (% DM) of Broiler basal diets

The chemical composition of broiler pre-starter, starter and finisher basal diets are presented in (Table 7). The percent CP, EE, CF, TA, AIA, NFE, calcium and phosphorus values of broiler pre starter, starter and finisher diets were 22.04, 5.37, 6.16, 10.40, 2.08, 56.03, 0.97 and 0.57; 21.50, 5.12, 6.98, 10.43, 2.15, 55.97, 1.0 and 0.55; 19.57, 5.18, 6.96, 10.56, 2.16, 57.73, 0.94 and 0.56, respectively. The calculated lysine and methionine content (%) of the basal diets were 1.2, 0.52 (pre-starter); 1.09, 0.48 (starter) and 0.97, 0.47 (finisher), respectively. The calculated ME (kcal/kg) was 3000, 3049, and 3099 for the pre-starter, starter and finisher diets, respectively.

Table 6: Chemical composition+ (%) of feed ingredients used in the experiment

Constituents	Ingredients				
	Maize	**Soybean meal**	**Fish meal**	**De oiled Rice Bran**	**Shrimp waste meal**
Dry matter	90.2	90.8	91.17	93.6	94.17
Crude protein	10.5	45.00	55.00	13.0	50.5
Ether extract	1.50	1.02	2.5	0.80	8.20
Crude fiber	6.7	9.46	2.4	16.8	15.2
Nitrogen free extract	75	36.28	19.6	53.9	7.0
Total ash	6.30	8.24	20.50	15.5	19.1
Calcium	0.01	0.20	6.50	0.06	6.0
Phosphorus	0.13	0.3	3.50	0.80	1.2
Lysine*	0.2	1.28	3.5	0.51	1.66
Methionine*	0.2	0.33	1.19	0.29	0.80
ME (k.cal /kg)**	3300	2300	2200	1400	1515

+ on dry matter basis except for DM

Source: * Feeding of poultry – Panda *et al.* (1984)

** Leeson and Summer (2008).

Table 7: Chemical composition (%) of broiler basal experimental diets on DMB

Nutrient	Basal diets		
	Pre-Starter	Starter	Finisher
Dry matter	90.1	90.36	91.06
Crude protein	22.04	21.50	19.57
Crude fat	5.37	5.12	5.18
Crude Fibre	6.16	6.98	6.96
Total ash	10.40	10.43	10.56
Acid insoluble ash	2.08	2.15	2.16
Nitrogen free extract	56.03	55.97	57.73
Calcium	0.97	1.0	0.94
Phosphorus	0.57	0.55	0.56
Lysine*	1.20	1.09	0.97
Methionine*	0.52	0.48	0.47
ME (kcal/kg)*	3000	3049	3099

* calculated values

4.2 *IN VIVO* STUDIES OF SHRIMP WASTE MEAL AND AMINOACIDS SUPPLEMENTATION ON BROILER PERFORMANCE

4.2.1 Body weight gain

4.2.1.1 Pre starter period (0-14 days)

Body weight gains during pre-starter period (0-14 days) are shown in the (table 8). The values of body weight gains found in the treatments T_1, T_2, T_3, T_4 and T_5 were 427.73, 416.6, 413.5, 425.23 and 417.16 g, respectively. The results revealed that there was no significant difference in body weight gain among treatments during pre-starter phase.

4.2.1.2 Starter period (15-28 days)

The body weight gains during the starter period (15-28 days) are presented in the table 8. Body weight gains in T_1, T_2, $T_{3,}$ T_4 and T_5 were observed to be 875.56, 889.13, 848.46, 898.73 and 853.56 g, respectively. Highest weight gain (898.73 g) was recorded in broilers fed with the diets containing 50% SWM protein along with synthetic lysine and methionine (T_4).Whereas the lowest weight gain (853.56 g) was observed in birds fed with the diet T_3.

4.2.1.3 Finisher period (29-42 days)

During the finisher phase, the body weight gains ranged between 823.16 g (T_3) to 887.26 g (T_4) among different treatments (Table 8). The results revealed significant differences in body weight gain among different treatments. Highest (887.26 g) and lowest (823.16 g) body weight gain were noticed in T_4 and T_3 groups, respectively. Significantly ($P<0.01$) higher body weight gains were noticed in the birds fed with the diets T_1, T_2 and T_4 when compared to T_3 and T_5.

4.2.1.4 Overall period (0-42 days)

During the entire growth phase, the body weight gains in T_1, T_2, T_3, T_4 and T_5 were found to be 2146.63, 2147.43, 2087.13, 2211.23 and 2101.66 g, respectively (Table 8). Diets containing 50% SWM protein along with synthetic lysine and methionine (T_4), T_2 (50% SWM protein) and T_1 (10% FM protein) showed significantly (P<0.01) higher weight gains when compared with T_3 (10% SWM protein) and T_5 (T_3+ synthetic lysine and methionine).

Table 8: Effect of supplementation of shrimp waste meal on body weight gain in broilers

Treatment	Weight gain (g)			
	Pre- Starter^{NS} (0-14 days)	Starter** (15-28 days)	Finisher** (29-42 days)	Total** (0-42 days)
T_1	427.73 ± 9.39	875.56^b ± 7.51	843.33^b ± 3.33	2146.63^b ±18.08
T_2	416.60 ± 2.91	889.13^a ± 1.37	841.7^{bc} ± 2.43	2147.43^b ± 1.88
T_3	413.50 ± 2.95	848.46^c ± 1.09	823.16^c ± 3.31	2087.13^c ± 4.25
T_4	425.23 ± 2.28	898.73^a ± 0.81	887.26^a ± 12.9	2211.23^a ± 12.2
T_5	417.16 ± 2.19	853.56^c ± 2.61	830.93^{bc} ± 2.34	2101.66^c ± 5.22

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.2 Feed intake

4.2.2.1 Pre starter period (0-14 days)

Feed intake during pre-starter period ranged from 632.8 (T_5) to 673.3 g (T_1) among different treatments (Table 9). Analysis of data revealed that there was a significant ($P<0.01$) increase in feed intake among the birds allotted to T_1 (control) when compared to birds fed diets from T_2 to T_5.

4.2.2.2 Starter period (15-28 days)

Feed intake during starter period ranged from 1290.97 (T_3) to 1319.4 g (T_2) among different treatments (Table 9). The results showed significantly ($P<0.01$) lower feed intake in $T_{1,}$ T_3 and T_5 when compared to T_2 and T_4 groups.

4.2.2.3 Finisher period (29-42 days)

Feed intake during finisher period ranged from 1865.96 (T_3) to 1890.7g (T_2) among different treatments (Table 9). The results revealed that there was no significant difference regarding feed intake among different treatment groups in finisher phase.

4.2.2.4 Overall period (0-42 days)

During the entire growth phase, the feed intake ranged from 3799.63 (T_3) to 3856.2g (T_2) among different treatments (Table 9). The overall effect of the phase revealed a non significant difference regarding feed intake among different treatment groups.

Table 9: Effect of supplementation of shrimp waste meal on feed intake in broilers

Treatment	Feed intake (g)			
	Pre- Starter** (0-14 days)	Starter** (15-28 days)	Finisher^{NS} (29-42 days)	Total^{NS} (0-42 days)
T_1	673.3^{a} ± 0.88	1297.8^{c} ± 1.32	1875.7 ± 53.4	3846.91 ± 54.8
T_2	646.13^{bc} ± 3.28	1319.4^{a} ± 5.64	1890.7 ± 11.63	3856.20 ± 18.13
T_3	642.7^{bc} ± 7.15	1290.97^{c} ± 1.04	1865.96 ± 1.93	3799.63 ± 4.49
T_4	650.2^{b} ± 5.26	1313.4^{ab} ± 7.05	1873.73 ± 4.39	3837.23 ± 6.87
T_5	632.8^{c} ± 6.44	1299.93^{bc} ± 4.04	1879.6 ± 6.27	3824.73 ± 9.11

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.3 Feed efficiency

4.2.3.1 Pre starter period (0-14 days)

During pre-starter phase the feed efficiency among treatments was not significantly different and the values were 1.57, 1.54, 1.55, 1.52, 1.51 in treatment T_1 to T_5 respectively (Table 10).

4.2.3.2 Starter period (15-28 days)

Feed efficiency during starter period ranged from 1.45 (T_4) to 1.52 (T_5) among different treatments (Table 10). The feed efficiency was significantly higher ($P<0.01$) in the birds fed with the diets containing T_1 , T_2 and T_4 than the birds fed with T_3 and T_5 .

4.2.3.3 Finisher period (29-42 days)

The values of feed efficiency during finisher phase for T_1, T_2, $T_{3,}$ T_4 and T_5 were found to be 2.2, 2.24, 2.26, 2.1, 2.27 respectively (Table 10). Statistical analysis on feed efficiency revealed non significant difference among treatments groups.

4.2.3.4 Overall period (0-42 days)

During the entire growth phase, the feed efficiency ranged from 1.87 (T_1) to 1.89 (T_2) among the treatments (Table 10).. The overall effect of the phase revealed a non significant difference regarding feed efficiency among different treatment groups.

Table 10: Effect of supplementation of shrimp waste meal on feed efficiency in broilers

Treatment	Feed efficiency			
	Pre- Starter^{NS} (0-14 days)	**Starter** (15-28 days)**	**Finisher^{NS} (29-42 days)**	**Total^{NS} (0-42 days)**
T_1	1.57 ± 0.03	1.48^{b} ± 0.015	2.2 ± 0.07	1.87 ± 53.4
T_2	1.54 ± 0.004	1.48^{b} ± 0.005	2.24 ± 0.02	1.89 ± 11.63
T_3	1.55 ± 0.014	1.516^{a} ± 0.003	2.26 ± 0.01	1.86 ± 1.93
T_4	1.52 ± 0.02	1.45^{b} ± 0.006	2.1 ± 0.03	1.87 ± 4.39
T_5	1.51 ± 0.02	1.52^{a} ± 0.005	2.27 ± 0.01	1.87 ± 6.27

^{ab} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.4 Digestibility of nutrients

4.2.4.1 Digestibility of nutrients during starter phase

The effect of experimental diets on digestibility of nutrients during starter phase is reported in Table 11. During the starter phase no significant difference was noticed among treatment groups regarding DM and CF digestibility (%) and the values were 66.7, 66.9, 66.7, 67.6 and 66.78 (DM) ; and 30.45, 30.24, 29.94, 30.7, 30.15 (CF) in birds fed on diets from T_1 to T_5 respectively.

The CP digestibility was significantly ($P<0.01$) higher in T_4 (70.25) than in other diets and the values were 68.23, 69.06, 66.78, 70.25, 67.05 in birds fed on diets from T_1 to T_5 respectively. The EE digestibility (%) values in birds fed on diets from T_1 to T_5 fed birds were 79.02, 79.23, 78.49, 79.72, 79.11 respectively and the EE digestibility was significantly ($P<0.01$) higher in birds fed with T_4 and T_2 compared to other diets.

4.2.4.2 Digestibility of nutrients during finisher phase

The effect of feeding experimental diets on digestibility of nutrients during finisher phase is reported in Table 12.

During the finisher phase the DM (%) digestibility was non significant among the treatments and the values were 70.77, 70.64, 70.47, 71.22 and 70.41 in birds fed T_1 to T_5, respectively. However, the CP (%) digestibility was significantly higher ($P<0.01$) in T_4 (69.51) fed birds than in other diets.

Table 11: Effect of supplementation of shrimp waste meal on digestibility of nutrients during starter phase

Treatment	Digestibility %			
	DM^{NS}	CP**	EE**	CF^{NS}
T_1	66.70 ± 0.32	68.23^{c} ± 0.10	79.02^{ab} ± 0.13	30.45 ± 0.09
T_2	66.90 ± 0.17	69.06^{b} ± 0.36	79.23^{a} ± 0.23	30.24 ± 0.15
T_3	66.70 ± 0.1	66.78^{d} ± 0.09	78.49^{b} ± 0.32	29.94 ± 0.23
T_4	67.60 ± 0.25	70.25^{a} ± 0.1	79.72^{a} ± 0.35	30.70 ± 0.06
T_5	66.78 ± 0.26	67.05^{d} ± 0.07	79.11^{ab} ± 0.12	30.15 ± 0.28

^{abcd} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

Table 12: Effect of supplementation of shrimp waste meal on digestibility of nutrients during Finisher phase

Treatment	Digestibility %			
	DM^{NS}	CP**	EE**	CF^{NS}
T_1	70.77 ± 0.2	68.58^{b} ± 0.22	77.9^{b} ± 0.21	31.49 ± 0.02
T_2	70.64 ± 0.04	68.99^{ab} ± 0.29	77.79^{b} ± 0.28	31.61 ± 0.06
T_3	70.47 ± 0.27	67.22^{c} ± 0.25	77.08^{c} ± 0.27	31.39 ± 0.09
T_4	71.22 ± 0.23	69.51^{a} ± 0.19	78.6^{a} ± 0.10	31.01 ± 0.33
T_5	70.41 ± 0.14	67.84^{c} ± 0.17	77.33^{bc} ± 0.21	31.19 ± 0.25

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

The EE digestibility (%) values for T_1 to T_5 fed birds were 77.9, 77.79, 77.08, 78.6 and 77.33 respectively and the EE digestibility was significantly (P<0.01) higher in birds fed with T_4 when compared to other diets. No significant difference was noticed among treatment groups regarding CF digestibility (%) and the values were 31.49, 31.61, 31.39, 31.01 and 31.19 in T_1 to T_5 fed birds respectively.

4.2.5 *E.coli* count of cecal contents

The effect of experimental diets on *E.coli* count (log cfu/g) of cecal content is presented in Table 13. During the pre-starter phase the *E.coli* count (log cfu/g) was significantly reduced in T_3 (5.62), T_5 (5.65) and T_4 (5.98) when compared to control T_1 (6.2) and T_2 (6.01). During the starter phase the *E.coli* count (log cfu/g) was significantly reduced in T_5 (5.25) and T_3 (5.26) when compared to control T_1 (5.88), T_2 (5.41) and T_4 (5.46).

The effect of experimental diets (Table 13) on the *E.coli* count (log cfu/g) of the cecal content showed a significant reduction (P<0.01) in *E.coli* count (log cfu/g) in diets (T_3, T_4 and T_5) when compared to control (T_1) and (T_2) from pre-starter to finisher phase.

4.2.6 *Salmonella* count of cecal contents

The effect of experimental diets on *Salmonella* count (log cfu/g) of cecal contents is presented in Table 13. During the pre-starter phase the *Salmonella* count (log cfu/g) of the cecal contents was significantly reduced (P<0.01) in birds fed diets containing T_3 (3.45) and T_5 (3.47) when compared to control T_1 (3.82).

During the starter phase the *Salmonella* count (log cfu/g) was significantly reduced in T_3 (3.22), T_5 (3.24) and T_4 (3.39) when compared to control T_1 (3.63) and T_2 (3.45). During the finisher phase the *Salmonella* count (log cfu/g) was significantly (P<0.01) reduced in T_3 (3.12) and T_5 (3.12) when compared to control T_1 (3.37)

The *Salmonella* count (log cfu/g) was significantly lower (P<0.01) in birds fed T_3, T_4 and T_5 diets when compared to control T_1 and T_2 irrespective of phase.

Table 13: Effect of supplementation of shrimp waste meal on gut pathogen population (*E.coli* and *Salmonella* count (log cfu/g) of cecal content)

	T_1	T_2	T_3	T_4	T_5
***E.coli* count**					
Pre-starter	$6.20^{a} \pm 0.03$	$6.01^{b} \pm 0.02$	$5.62^{c} \pm 0.46$	$5.98^{b} \pm 0.03$	$5.65^{c} \pm 0.07$
Starter	$5.88^{a} \pm 0.1$	$5.41^{b} \pm 0.02$	$5.26^{c} \pm 0.02$	$5.46^{b} \pm 0.03$	$5.25^{c} \pm 0.03$
Finisher	$5.86^{a} \pm 0.02$	$5.23^{b} \pm 0.01$	$5.13^{c} \pm 0 .01$	$5.21^{b} \pm 0.01$	$5.15^{c} \pm 0.01$
***Salmonella* count**					
Pre-starter	$3.82^{a} \pm 0.02$	$3.56^{b} \pm 0.02$	$3.45^{d} \pm 0.01$	$3.53^{bc} \pm 0.02$	$3.47^{cd} \pm 0.01$
Starter	$3.63^{a} \pm 0.02$	$3.45^{b} \pm 0.02$	$3.22^{d} \pm 0.01$	$3.39^{c} \pm 0.01$	$3.24^{d} \pm 0.01$
Finisher	$3.37^{a} \pm 0.017$	$3.17^{b} \pm 0.01$	$3.12^{c} \pm 0.01$	$3.18^{b} \pm 0.01$	$3.12^{c} \pm 0.02$

^{abcd} Values in a row not sharing common superscripts differ significantly **(P<0.01)

4.2.7 Serological parameters during pre-starter phase (At the end of two weeks of age)

4.2.7.1 Serum total protein

The effect of feeding experimental diets on serum total protein (g/dl) is reported in Table 14. Serum total protein values of birds (g/dl) reared on diets T_1, T_2, T_3, T_4 and T_5 were 4.08, 4.26, 4.25, 4.36 and 4.56 respectively. Statistical analysis revealed non-Significant difference among treatment groups.

4.2.7.2 Serum albumin and globulin

The effect of feeding experimental diets on serum albumin and globulin (g/dl) is reported in Table 14. Serum albumin values of birds (g/dl) reared on diets T_1, T_2, T_3, T_4 and T_5 were 2.56, 2.58, 2.72, 2.7 and 2.64 respectively. No significant difference was noticed among experimental diets.

Similar trend was observed for serum globulins also. No significant difference was observed among experimental groups.

4.2.7.3 Serum cholesterol

The effect of feeding experimental diets on serum cholesterol (mg/dl) is reported in Table 14. Serum cholesterol values of birds (mg/dl) reared on diets T_1, T_2, T_3, T_4 and T_5 were 112.99, 116, 113.44, 114.34 and 115.65, respectively. No significant difference was noticed among experimental diets.

4.2.7.4 Serum glucose

The effect of feeding experimental diets on serum glucose (mg/dl) is reported in Table 14. Serum glucose values of birds (mg/dl) reared on diets T_1, T_2, T_3, T_4 and T_5 were 379.02, 378.03, 376.52, 374.83 and 371.5 respectively. No significant difference was noticed among experimental diets.

Table 14: Effect of supplementation of shrimp waste meal on serum parameters during pre-starter phase (At the end of 2 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin^{NS} (g/dl)	Cholesterol^{NS} (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.08 ± 0.11	2.56 ± 0.06	1.52 ± 0.07	112.99 ± 0.59	379.02±2.29
T_2	4.26 ± 0.05	2.58 ± 0.05	1.68 ± 0.07	116.0 ± 0.58	378.03±0.99
T_3	4.25 ± 0.07	2.72 ± 0.02	1.52 ± 0.06	113.44 ± 1.04	376.52±0.81
T_4	4.36 ± 0.06	2.7 ± 0.02	1.66 ± 0.06	114.34 ± 0.85	374.83±0.94
T_5	4.56 ± 0.05	2.64 ± 0.04	1.72 ± 0.08	115.65 ± 1.08	371.5±0.76

^{ab} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.8 Serological parameters during Starter phase (At the end of four Weeks of age)

4.2.8.1 Serum total protein

The effect of feeding experimental diets on serum total protein (g/dl) during starter phase is presented in Table 15.

Serum total protein values of birds (g/dl) reared on diets T_1, T_2, T_3 ,T_4 and T_5 after 4 weeks were 4.43, 4.5, 4.56, 4.71 and 4.35, respectively. The serum total protein (g/dl) values were not significantly different among treatments.

4.2.8.2 Serum albumin and globulin

The effect of feeding experimental diets on serum albumin and globulin (g/dl) values were reported in Table 15.

Serum albumin values of birds (g/dl) reared on diets T_1, T_2, $T_{3,}T_4$ and T_5 after 4 weeks were 2.56, 2.46, 2.75, 2.58 and 2.57, respectively. No significant difference was noticed among experimental diets.

Serum globulin values of birds (g/dl) reared on diets T_1, T_2, $T_{3\,,}T_4$ and T_5 after 4 weeks were 1.93, 2.03, 1.81, 2.13 and 1.77, respectively (Table 15). Statistical analysis revealed non-Significant difference among treatment groups.

4.2.8.3 Serum cholesterol

Serum Cholesterol values of birds (mg/dl) reared on diets T_1, T_2, $T_{3,}T_4$ and T_5 after 4 weeks were 116.43, 118.53, 118.36, 116.58 and 117.51, respectively. No significant difference was noticed among experimental diets.

4.2.8.4 Serum glucose

Serum glucose values of birds (mg/dl) reared on diets T_1, T_2, T_3, T_4 and T_5 after 4 weeks were 285.5, 284.33, 284.83, 284.16 and 284.21, respectively. No significant difference was noticed among experimental diets.

Table 15: Effect of supplementation of shrimp waste meal on serum parameters during starter phase (At the end of 4 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin^{NS} (g/dl)	Cholesterol^{NS} (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.43 ± 0.06	2.56 ± 0.08	1.93 ± 0.09	116.43 ± 1.05	285.5 ± 2.52
T_2	4.50 ± 0.14	2.46 ± 0.03	2.03 ± 0.15	118.53 ± 0.9	284.33 ± 3.45
T_3	4.56 ± 0.1	2.75 ± 0.04	1.81 ± 0.11	118.36 ± 1.7	284.83 ± 2.72
T_4	4.71 ± 0.13	2.58 ± 0.06	2.13 ± 0.13	116.58 ± 1.38	284.16 ± 3.78
T_5	4.35 ± 0.1	2.57 ± 0.08	1.77 ± 0.09	117.51 ± 0.68	284.21 ± 1.05

NS- Non-significant

4.2.9 Serological parameters during finisher phase (At the end of six weeks of age)

4.2.9.1 Serum total protein

The effect of feeding experimental diets on serum total protein (g/dl) at the end of 6 weeks is presented in Table 16. Serum total protein values of birds (g/dl) reared on diets T_1, T_2, T_3 ,T_4 and T_5 after 6 weeks were 4.58, 4.63, 4.31, 4.55 and 4.28, respectively. Statistical analysis revealed non-Significant difference among treatment groups.

4.2.9.2 Serum albumin and globulin

The effect of feeding experimental diets on serum albumin and globulin (g/dl) values at the end of 6 weeks was reported in Table 16.

Serum albumin values of birds (g/dl) reared on diets T_1, T_2, T_3,T_4 and T_5 after 6 weeks were 2.68, 2.75, 2.63, 2.58 and 2.8 respectively. No significant differences were noticed among experimental diets.

Serum globulin values of birds (g/dl) reared on diets T_1, T_2, T_3,T_4 and T_5 after 6 weeks were 2.06, 1.71, 1.41, 1.5 and 1.61, respectively. Statistical analysis revealed Significant ($P<0.01$) differences in serum globulin values (g/dl) among different treatments (Table 16). Serum globulin values were significantly higher in birds fed control diet (T_1) than in other treatments.

4.2.9.3 Serum cholesterol

Serum cholesterol values of birds (mg/dl) reared on diets T_1, T_2, $T_{3,}T_4$ and T_5 after 6 weeks were 120.85, 113.65, 111.26, 115.58 and 113.78, respectively. The serum cholesterol (mg/dl) was significantly higher (P<0.01) in T_1 than in than in other treatments

4.2.9.4 Serum glucose

Serum glucose values of birds (mg/dl) reared on diets T_1, T_2, $T_{3,}T_4$ and T_5 after 6 weeks were 373.75, 383.22, 386.36, 380.43 and 386.23, respectively. No significant differences were noticed among experimental diets.

Table 16: Effect of supplementation of shrimp waste meal on serum parameters during finisher phase (At the end of 6 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin** (g/dl)	Cholesterol** (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.58 ± 0.08	2.68 ± 0.04	2.06^{a} ± 0.19	120.85^{a} ± 0.84	373.75 ± 10.9
T_2	4.63 ± 0.06	2.75 ± 0.02	1.71^{ab} ± 0.13	113.65^{bc} ± 1.64	383.22 ± 2.82
T_3	4.31 ± 0.13	2.63 ± 0.02	1.41^{b} ± 0.08	111.26^{c} ± 1.75	386.36 ± 1.73
T_4	4.55 ± 0.12	2.58 ± 0.06	1.5^{b} ± 0.16	115.58^{b} ± 1.38	380.43 ± 2.58
T_5	4.28 ± 0.1	2.81 ± 0.03	1.61^{b} ± 0.07	113.78^{bc} ± 1.28	386.23 ± 1.93

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.10 BLOOD CELLS

4.2.10.1 RBC count

The effect of experimental diets on RBC count (10^6/ µl) is presented in Table 17. Statistical analysis on RBC count revealed non significant differences among treatment groups during the three phases of the study and the values ranged from 2.16 (T_3) to 2.47 (T_5) in pre-starter phase; 3.08 (T_5) to 3.61 (T_4) in starter phase and 2.30 (T_1) to 2.87 (T_4) in finisher phase.

4.2.10.2 WBC count

The effect of experimental diets on WBC count (10^3/ µl) is presented in Table 18. During pre-starter phase the WBC count (10^3/µl) was significantly higher ($P<0.01$) in the birds fed with the diets T_2, T_3, T_4 and T_5 when compared to the birds fed with T_1 control diet. During starter and finisher phases the WBC count (10^3/µl) was significantly higher ($P<0.01$) in the birds fed with the diets T_2 and T_3 than the birds fed with T_1 , T_4 and T_5 .

4.2.10.3 Lymphocyte (%)

The effect of experimental diets on lymphocyte (%) is presented in Table 19. During the pre-starter and starter phases lymphocyte (%) was significantly higher ($P<0.01$) in T_2 (58.89), T_3 (58.31) than in T_1 (57.47), T_4 (57.43) and T_5 (57.63) fed birds. whereas during the finisher phase there was no significant difference among treatments.

Table 17: Effect of supplementation of shrimp waste meal on RBC count ($10^6/\mu l$)

Phases	T1	T2	T3	T4	T5
Pre-starter NS	2.20 ± 0.05	2.29 ± 0.12	2.16 ± 0.08	2.39 ± 0.2	2.47 ± 0.24
Starter NS	3.10 ± 0.31	3.38 ± 0.14	3.35 ± 0.16	3.61 ± 0.07	3.08 ± 0.18
Finisher NS	2.36 ± 0.14	2.55 ± 0.21	2.59 ± 0.14	2.87 ± 0.07	2.71 ± 0.07

NS- Non-significant

Table 18: Effect of supplementation of shrimp waste meal on WBC count ($10^3/\mu l$)

Phases	T1	T2	T3	T4	T5
Pre-starter **	27.65^b±0.34	29.6^a ± 0.22	29.86^a ± 0.20	29.22^a± 0.25	29.12^a± 0.35
Starter **	29.4^c ± 0.17	30.26^a±0.03	30.07^{ab}±0.09	29.79^b± 0.06	29.9^b ± 0.09
Finisher **	29.48^c±0.23	30.22^a±0.06	29.94^{ab}±0.12	29.82^{bc}±0.05	29.82^{bc}± 0.05

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

Table 19: Effect of supplementation of shrimp waste meal on lymphocyte (%)

Phases	T1	T2	T3	T4	T5
Pre-starter**	56.68^c±0.27	60.04^{ab}±0.55	61.17^a± 0.12	58.7^b ± 1.11	58.16^{bc}±0.68
Starter **	57.47^b± 0.31	58.89^a± 0.28	58.31^{ab}±0.32	57.43^b ± 0.3	57.63^b± 0.39
Finisher NS	68.21 ± 0.6	67.25 ± 0.83	68.75 ± 0.57	68.17± 0.37	67.63 ± 0.4

^{abc} Values in a row not sharing common superscripts differ significantly **(P<0.01)

NS- Non-significant

4.2.11 Carcass characteristics

The live weight of birds at slaughter (table 20) in T_1 to T_5 treatments were in the range of 1.81 (T_3) to 2.21 kg (T_4). Birds fed T_4 diets had significantly higher value when compared to T_3 and T_5 group.

A similar trend was observed regarding hot carcass weight. Significantly ($P<0.01$) higher values were recorded in birds fed with T_4 diet when compared to other treatments.

The dressing (%) values in experimental diets T_1, T_2, $T_{3,}$ T_4 and T_5 were 65.51, 66.83, 63.51, 67.68 and 64.32, respectively. Statistical analysis of data revealed that the birds fed T_4 diet had significantly higher dressing percentage when compared to birds fed T_1, T_2, T_3 and T_5 diets.

The liver weight of the birds at slaughter (table 20) in T_1 to T_5 treatments were in the range of 43.38g (T_3) to 46.78g (T_4). Birds fed $T_{4,}$ $T_{2,}$ and T_1 diets had significantly higher values when compared to T_3 and T_5 group.

The gizzard weight (g) in experimental diets T_1, T_2, $T_{3,}$ T_4 and T_5 were 36.83, 39.72, 34.58, 40.02 and 34.07, respectively. Statistical analysis of data revealed that the birds fed T_4 and T_2, diets had significantly higher weights when compared to birds fed T_1, T_3 and T_5 diets.

The heart weight (g) of birds at slaughter in T_1 to T_5 treatments were in the range of 8.96 (T_5) to 10.27 (T_4). Statistical analysis of data revealed that the birds fed $T_{4,}$ T_1 and T_2 diets had significantly higher weights when compared to birds fed, T_3 and T_5 diets.

Table 20: Effect of supplementation of shrimp waste meal on carcass characteristics of broilers

Treatment	Live wt.** (kg)	Hot carcass wt.** (kg)	Dressing ** (%)	Liver wt.** (g)	Gizzard wt.** (g)	Heart wt.** (g)
T_1	$2.09^{b} \pm 0.03$	$1.36^{ab} \pm 0.02$	$65.51^{c} \pm 0.20$	$45.86^{a} \pm 0.99$	$36.83^{b} \pm 0.90$	$9.98^{ab} \pm 0.25$
T_2	$2.08^{b} \pm 0.04$	$1.39^{ab} \pm 0.03$	$66.83^{b} \pm 0.35$	$46.01^{a} \pm 0.56$	$39.72^{a} \pm 0.56$	$9.69^{abc} \pm 0.35$
T_3	$1.81^{c} \pm 0.06$	$0.98^{c} \pm 0.18$	$63.51^{d} \pm 0.12$	$43.38^{b} \pm 0.55$	$34.58^{bc} \pm 1.32$	$9.27^{bc} \pm 0.08$
T_4	$2.21^{a} \pm 0.04$	$1.49^{a} \pm 0.03$	$67.68^{a} \pm 0.13$	$46.78^{a} \pm 0.63$	$40.02^{a} \pm 0.61$	$10.27^{a} \pm 0.39$
T_5	$1.90^{c} \pm 0.05$	$1.21^{bc} \pm 0.04$	$64.32^{e} \pm 0.2$	$44.95^{ab} \pm 0.64$	$34.07^{c} \pm 0.57$	$8.96^{c} \pm 0.1$

^{abcd} Values in a row not sharing common superscripts differ significantly **($P<0.01$)

4.2.12 Cost economics

Statistical analysis revealed significant ($P<0.01$) differences in the feed cost per kg live weight gain among different treatments (Table 21). The feed cost per kg live weight gain ranged from Rs.48.08 (T_4) to Rs. 51.13 (T_5). The feed cost per kg live weight gain was significantly ($P<0.01$) low in birds fed diets (T_1, T_2 and T_4) when compared to T_3 and T_5. However the values among birds allotted to T_1, T_2 and T_4 groups were found to be comparable.

Table 21: Effect of supplementation of shrimp waste meal on cost economics of broiler production

Treatment	Feed intake per bird for 6 weeks ^{NS} (g)	Cost of feed/ bird ^{NS} (Rs)	Weight gain(g)**	Feed cost per kg live weight gain ** (Rs)
T_1	3846.03± 54.93	105.42 ± 1.28	2146.63^{b} ± 18.08	49.12^{bc} ± 0.76
T_2	3848.66± 15.16	105.9 ± 0.5	2147.43^{b} ± 1.88	49.32^{bc} ± 0.18
T_3	3798.02 ± 4.5	104.09 ± 0.96	2087.13^{c} ± 4.25	49.87^{ab} ± 0.36
T_4	3832.33 ± 9.2	106.29 ± 0.27	2211.23^{a} ± 12.22	48.08^{c} ± 0.25
T_5	3823.33 ± 9.27	107.45 ± 0.51	2101.66^{c} ± 5.28	51.13^{a} ± 0.35

^{abc} Values in a row not sharing common superscripts differ significantly
**(P<0.01)

4.2.13 Mortality

During pre-starter phase 0,2,0,1 and 1, during starter phase 0,2,2,5 and 4 and 2,1,1,2 and 3 birds during finisher phase have died in treatments T_1 to T_5, respectively during the experimental period. The causes of mortality as per postmortem reports include coccidiosis, pale liver, fatty liver etc.

Table 22: Effect of supplementation of shrimp waste meal on mortality (%) of broilers

Treatment	Mortality (%)		
	Pre-Starter	**Starter**	**Finisher**
T_1	0	0	2.66
T_2	2.66	2.66	1.66
T_3	0	2.66	1.33
T_4	1.33	5.33	2.66
T_5	1.33	4.0	3.07

CHAPTER - V

DISCUSSION

This chapter deals with the reasoning and due justification for the results obtained in the present work in conjunction with the observations of various authors and their publications relevant to performance of broilers fed shrimp waste meal.

5.1 Chemical composition of shrimp waste meal

The Shrimp waste meal procured from OM SAI AQUA, Venkateswarapuram contained 94.17% DM, 50.5 % CP, 8.2 % EE, 6.0 % Ca, 1.2% P, 15.2% CF , 19.1 % TA and 7% NFE (Table 6). The chemical composition of shrimp waste meal as reported by various authors is presented in table 1.

The CP content of SWM in the present study was 50.5 % which is as per the values reported by Rosenfeld *et al.,* (1997) on contrary lower CP values were reported by Okonkwo *et al.,* (2012), Mahata *et al*., (2008). However the EE, TA, CF, Ca and P content of SWM in the present study, when compared with the values reported by Rosenfeld *et al*.,(1997) were 8.2 vs. 6.31; 19.1 vs. 15.64; 15.2 vs. 8.92; 6.0 vs.5.21 and 1.2 vs. 1.47, respectively. The EE, CF, TA values of SWM used in the present study were higher than those reported by Rosenfeld *et al*., (1997).

This variation among different studies may be due to the difference in shrimp species (Ngoan *et al.,* 2000; Heu *et al.,* 2003), source of shrimp meal (head/shell) (Meyers, 1986) and/or processing method, as these can affect the nutritional values of SWM. Shrimp waste meal made from heads contains more CP and EE, less CF and TA than shrimp shells (Meyers *et al*., 1973).

5.2 Chemical composition (%) of feed ingredients

The chemical composition of maize, soybean meal, fish meal, DORB used in formulation of the experimental diets is given in the (Table 6).The CP content of maize used in the present study was 10.5% which is higher than the value (10.0) reported by Arora and Harjit kaur, (2010). The CP content of soybean meal (45.0%) , fish meal (55.0%) and DORB (13.0%) used in the present study were lower than the values given by Arora and Harjit Kaur, (2010) which were 51.0, 71.4 and 16.1, respectively. This may be due to variations in soil texture, season of harvesting and varieties of the feed ingredients.

5.3 EXPERIMENTAL DIETS

A basal pre starter, starter and finisher diets (Table 7) were prepared as per the nutrient requirements of the poultry, ICAR (2013) using maize, soybean meal, fish meal and DORB as these are the most commonly available ingredients in this region.

In pre-starter phase five experimental diets (Table 2) were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 20% level (T_2) , 30% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. In starter and finisher phases five experimental diets (Table 2.1) were prepared by replacing fish meal protein of the basal diet with the Shrimp waste meal protein at 50% level (T_2), 100% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB, and 10 % fish meal. All diets were iso-nitrogenous and iso-caloric. The level of incorporation of SWM during pre-starter phase was restricted to 20 and 30% as the birds during pre-starter phase cannot tolerate higher levels of chitin in the diet.

5.3.1 Chemical composition (%) of experimental diets

The experimental diets were formulated based on the recommendations of Nutrient requirements of poultry, ICAR (2013) suitable to the Indian conditions as per the latest revised research. The chemical composition of broiler pre-starter, starter, finisher experimental diets is shown in Table 3, 4 and 5, respectively. All the five diets during each phase were made iso-nitrogenous and iso-caloric and other ingredients like palm oil, L-Lysine and DL-Methionine were also used to meet the dietary concentration for energy, protein, lysine and methionine as per ICAR (2013) specifications for broiler diets. The CF and AIA content of the basal diets was within the permissible limits of BIS (1992). The synthetic lysine and methionine were added to the T_4 and T_5 diets as the SWM was having these amino acids in lesser amounts when compared to FM.

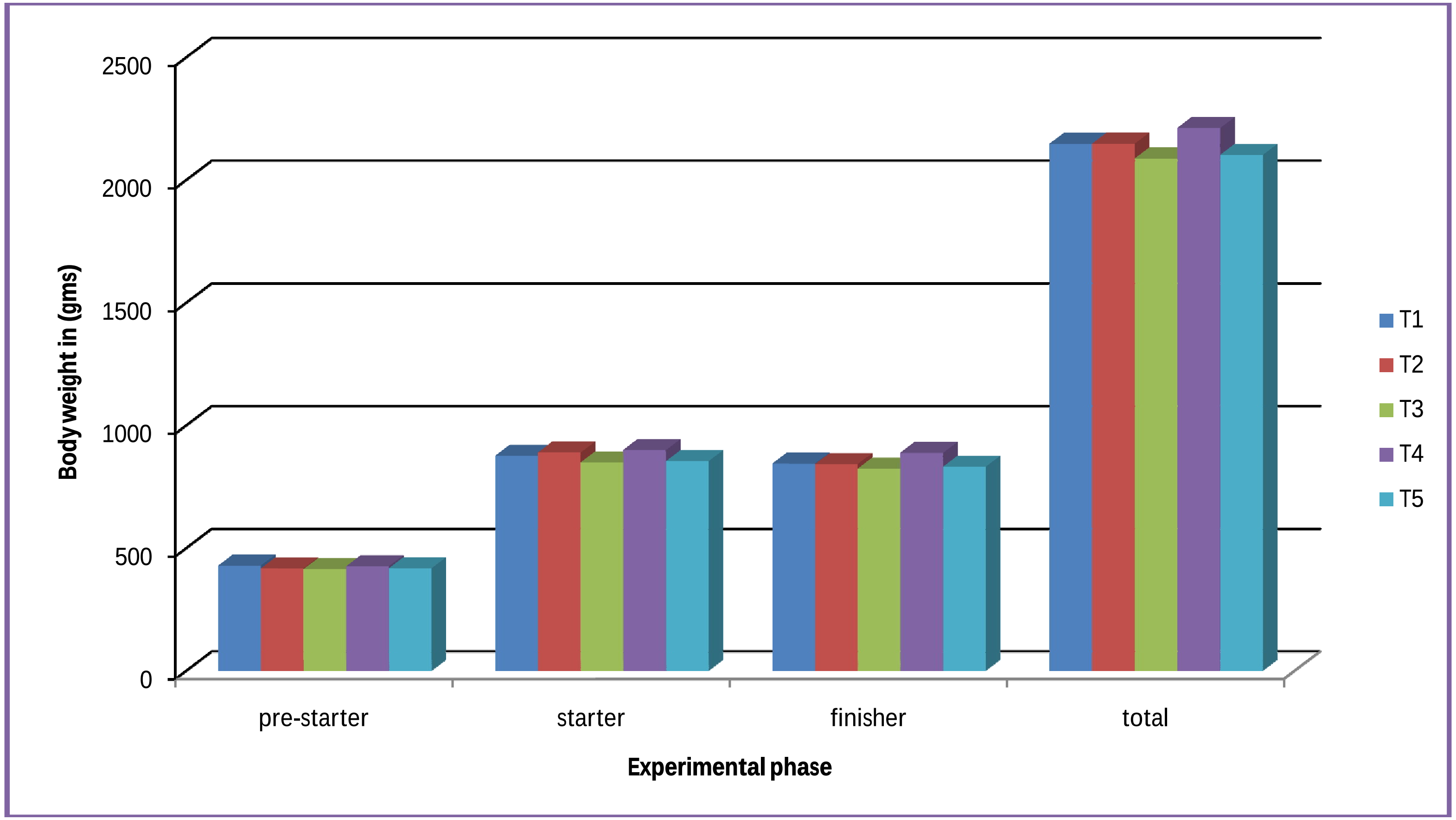
5.4 IN VIVO BROILER STUDIES

5.4.1 Body weight gain

Body weight gain of broilers during different phases and overall growth performance is presented in (Table 8 and Fig: 1). During the pre-starter phase, the body weight gain (BWG) values ranged from 413.5g (T_3) to 427.73g (T_1) and no significant difference was noticed among different treatment groups in this phase. During starter, finisher and overall growth phases birds fed with T_4 diet showed superior weight gain ($p<0.01$) compared to T_1 and T_2, whereas lowest weight gain was reported in the birds fed with T_3 and T_5 . These results were in congruence with the findings of Khempaka *et al* (2011)., who reported better weight gains at diets containing 5% SWM.

The increased body weight gains at 50% replacement of FM protein with SWM protein along with the supplementation of synthetic lysine and methionine were due to improved utilization of SWM protein as reported by Fanimo *et al.,* (1996). Decreased weight gain seen in the birds fed with diets in which FM protein was completely replaced with SWM protein might be due to the high fibre and chitin in SWM which may have caused an imbalance in the levels of amino acids in the diet and the sparing effect of one amino acid upon the other, as reported by Aron (1985). This may have been reduced by the supplemented synthetic amino acids since they are easily absorbed, and so this led to the better results when SWM was supplemented with synthetic amino acids.

Fig.1 Effect of supplementation of shrimp waste meal on Body weight gain (gms) of broilers.



5.4.2 Feed intake

In the present study the feed intake during pre-starter phase was reduced in the birds fed on diets with SWM when compared to control. This might be due to the less palatability of SWM by the young birds. The feed intake during starter phase was significantly more in birds fed with diets containing SWM at 50% replacement of FM than the (control/100% FM). This might be due to inferior energy utilization of SWM than FM and to meet the energy requirements the birds might have consumed more feed. The reduced feed intake of birds fed on 100% replacement of FM with SWM in T_3 and T_5 can be attributed to inferior palatability of SWM when compared to FM.

5.4.3 Feed Efficiency

During starter phase, feed efficiency differed significantly ($p<0.01$) among different treatments. No significant difference was noticed among treatments in pre starter, finisher and overall growth phases. Damron *et al*., (1964); Raab *et al*., (1971); Ojewola and Annah, (2006); Khempaka *et al*., (2011) also reported non significant differences among different treatments regarding feed efficiency.

In all the phases better feed efficiency was observed in the group fed with the diet containing 50% fish meal protein replaced with shrimp waste meal protein along with the synthetic amino acids supplementation due to the improved utilization of SWM protein than FM protein with the synthetic amino acids supplementation. But, in contrast Ingweye *et al.,* (2008) reported that FCR was best at 0% level of replacement and higher values were recorded for 100% replacement level.

Fig.2 Effect of supplementation of shrimp waste meal on Feed intake (gms) of broilers.

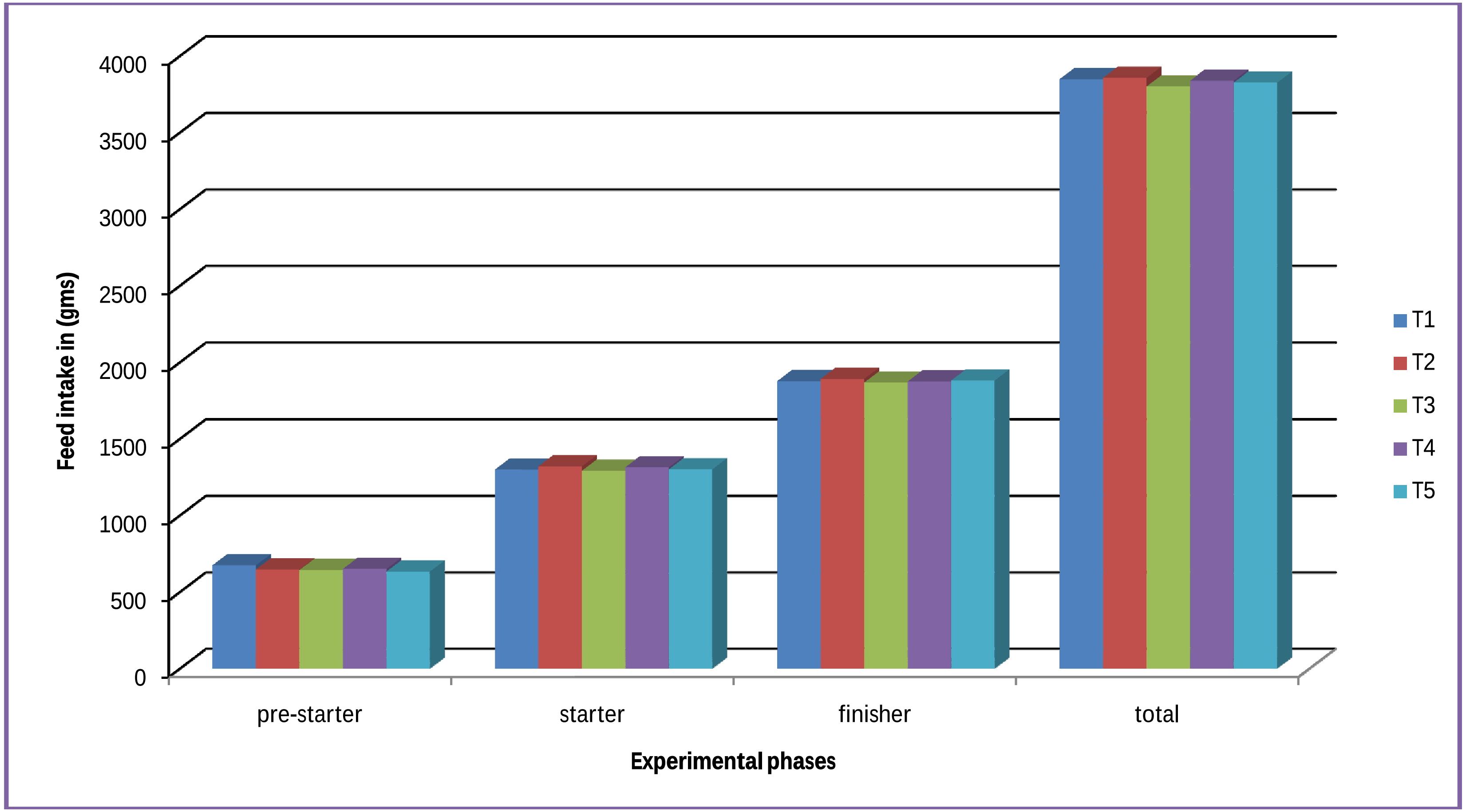
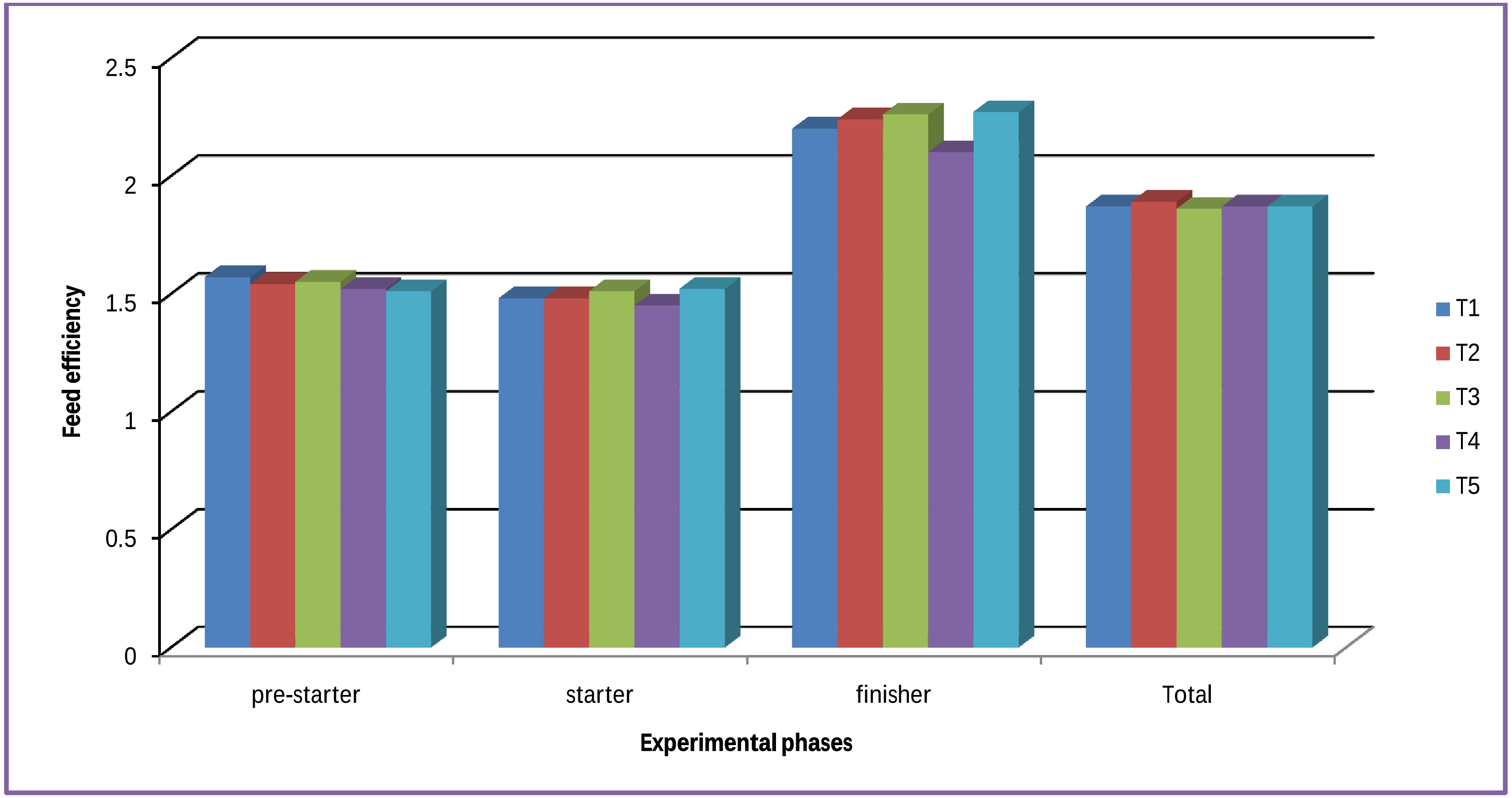


Fig.3 Effect of supplementation of shrimp waste meal on Feed efficiency in broilers.



5.4.4 Nutrient digestibility

During starter phase and finisher phases no significant differences were noticed regarding the digestibility of DM and CF among treatments (Table 11 and 12,). These findings were on line with Khempaka *et al.*, (2011) who reported that DM, OM and Ash digestibility values and nitrogen retention did not change significantly when SWM was at or below 15%. Ojewola and Annah, (2006) also reported non significant differences regarding fat, ash, crude fibre digestibility and nitrogen retention among treatment groups fed with SWM.

During starter and finisher phases the digestibility of CP and EE was significantly ($P<0.01$) higher in birds fed T_4 ration (50% SWM protein + Amino acids) and lower values were observed with the rations in which fish meal protein was completely substituted with SWM protein. Similar findings were reported by Mahata *et al.*, (2008) that there was a significant decrease in the nitrogen retention when SWH was used beyond 8% level of inclusion in the diet. The enhanced nutrient digestibility in birds fed T_4 ration may be due to reduced number of pathogenic bacteria *Escherichia coli, Salmonella typhimurium* (Choi *et al.*, 1994; LeM- ieux *et al.*, 2003; Wang *et al.*, 2003), increased immune function (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003), better secretion of digestive enzymes (Hou and Gao, 2001), better absorption of nutrients (Wu, 1998 and Huang *et al.*, 2005), (Li *et al.*, 2007 and Khambualai *et al.*, 2008 and 2009) .

The exoskeleton of the shrimp is composed mainly of chitin, an N-acetylated glucosamine polysaccharide that forms part of the protein complex, and is considered to have low digestibility when fed to animals (Austin *et al.*, 1981). Inclusion of SWM at higher levels in broiler diets causes due increase in chitin levels and it physically blocks the access of digestive enzymes to lipids and proteins, thus affecting the utilization of these nutrients (Castro *et al.*, 1989; Karasov, 1990).

5.4.5 ***E.coli* and *Salmonella* count**

Irrespective of the growth phase during entire experimental period there was a significant (P<0.01) reduction in the *E.coli* count (log cfu/g) (Table 13 and Fig:4) and *Salmonella* count (log cfu/g) (Table 13 and Fig:5) of cecal contents of broilers. The *E.coli* and *Salmonella* count of cecal contents decreased significantly (P<0.01) with increase in SWM in the diet.

The results of the present study were in good agreement with the findings of Khempaka *et al.*, (2011) who stated that feeding broilers with SWM resulted in increased population of lacto bacillus and decreased intestinal Escherichia coli and cecal salmonella count. Li *et al.*, (2007) also reported that the concentration of *E.coli* in the caecum was significantly decreased by dietary supplementation of 100 mg/kg of chiti-oligosaccharides in comparison with the control birds.

The observations of present study reinforce the claims of previous authors Menconi *et al.*, (2014), Chen *et al.*, (2002) and Vishu kumar *et al.*, (2005) that feeding of low levels of SWM containing chitin (or) low levels extracted chitin-chitosan were effective as pre-biotic in reducing the gut pathogen particularly *E.coli* and *Salmonella.* Menconi *et al*., (2014) reported a significant reduction in *Salmonella Typhimurium* count (log cfu/g) of cecal content in broilers fed chitosan at 0.2% and the values were 4.2 vs. 2.28 (trail 1) and 5 vs. 3.34 (trail 2) in control vs. chitosan fed diets.

Fig.4 Effect of supplementation of shrimp waste meal on *E.coli* count (log cfu/g) of cecal contents of broilers

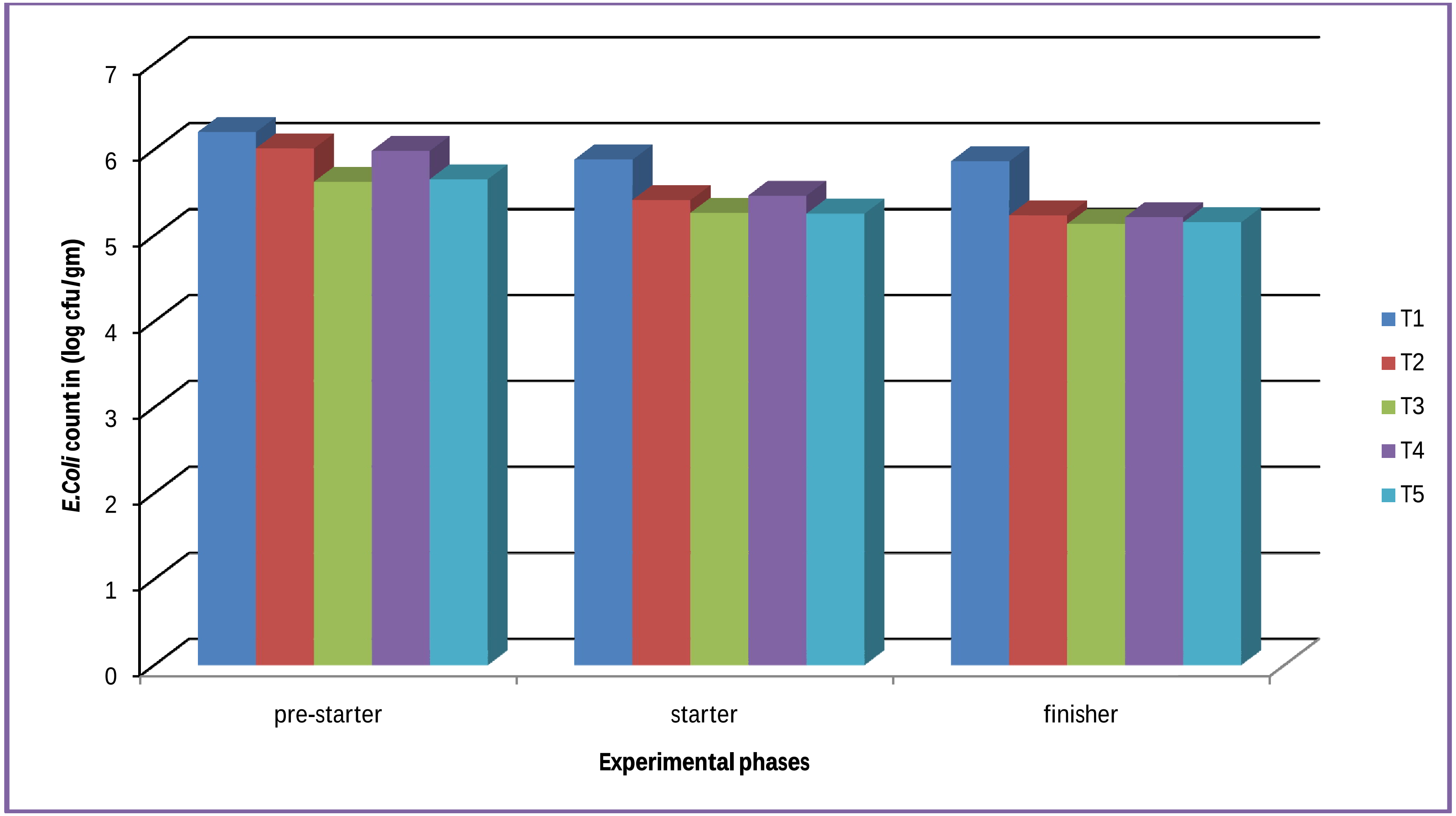
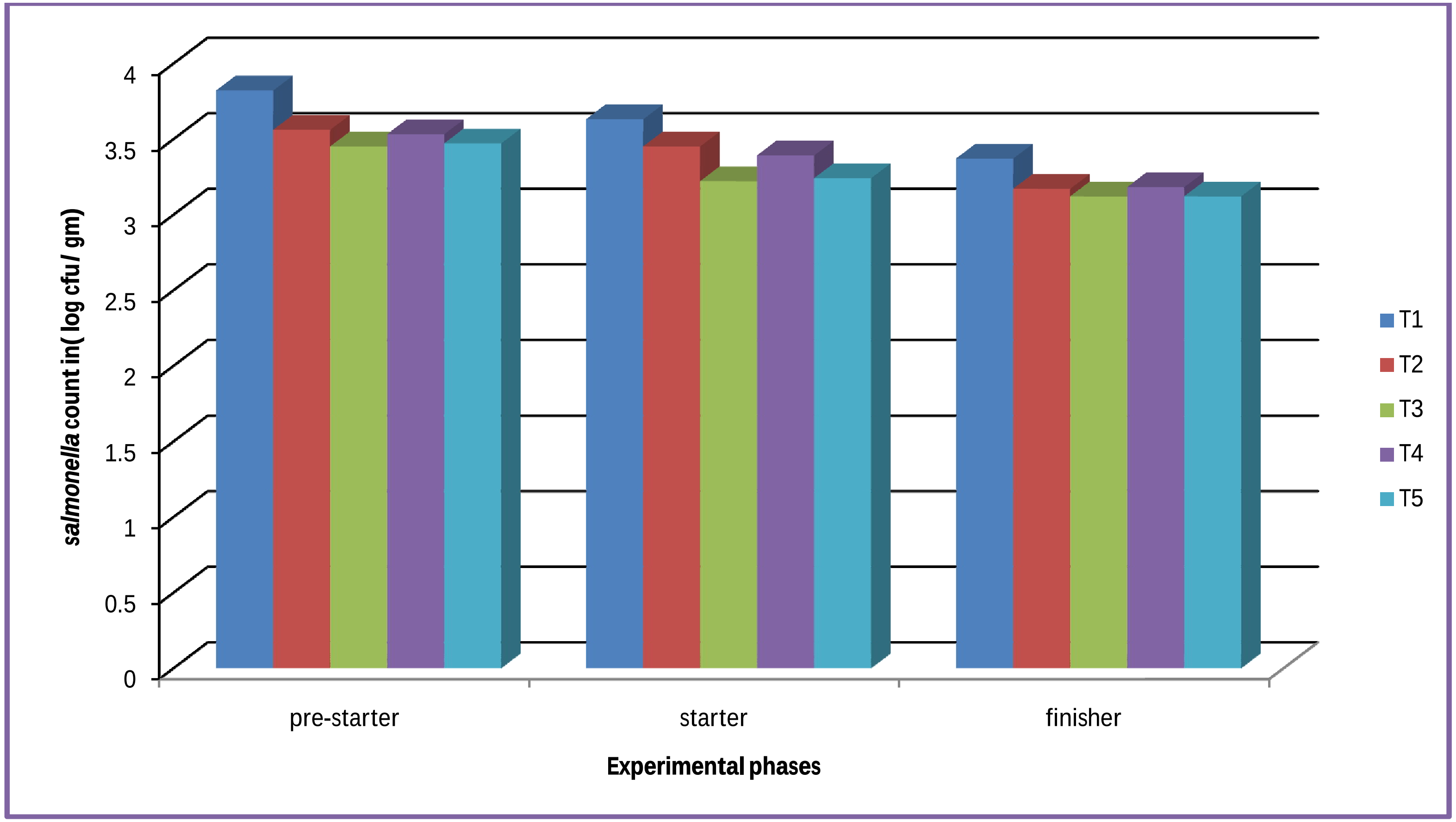


Fig.5 Effect of supplementation of shrimp waste meal on *Salmonella* count (log cfu/g) of cecal contents of broilers



5.4.6 Serological parameters

5.4.6.1 Serum total protein

No significant differences were observed in serum total protein content (Table 14,15 and 16, Fig : 6) among treatment groups during pre-starter, starter and finisher phases but, there was a slight increase in the serum total protein content in the groups fed with SWM diets when compared to control. Similar to the results of present study, earlier studies of Li *et al*., (2007) who reported that higher serum total protein content was observed when broilers were supplemented with chito-oligosaccharide at 100 mg/kg level than other treatment birds. Jabbal *et al*., (1998); Wang, (1998); Tang *et al*., (2005) also claimed that there was an increase in the level of serum total protein in pigs supplemented with chito-oligosaccharides.

5.4.6.2 Serum albumin and globulin

There was no significant difference regarding serum albumin values among treatment groups during pre-starter, starter and finisher phases (Table 14, 15 and 16 and Fig: 7, 8). The results are in consonance with the findings of Olayemi, (2001) who reported that there was no significant difference regarding serum metabolites among dietary treatments. No significant differences were noticed in serum globulin contents of different treatments during pre-starter and starter phases. But, the values differed significantly ($P<0.01$) in finisher phase.

Fig.6 Effect of supplementation of shrimp waste meal on Total protein (g/dl) of broilers

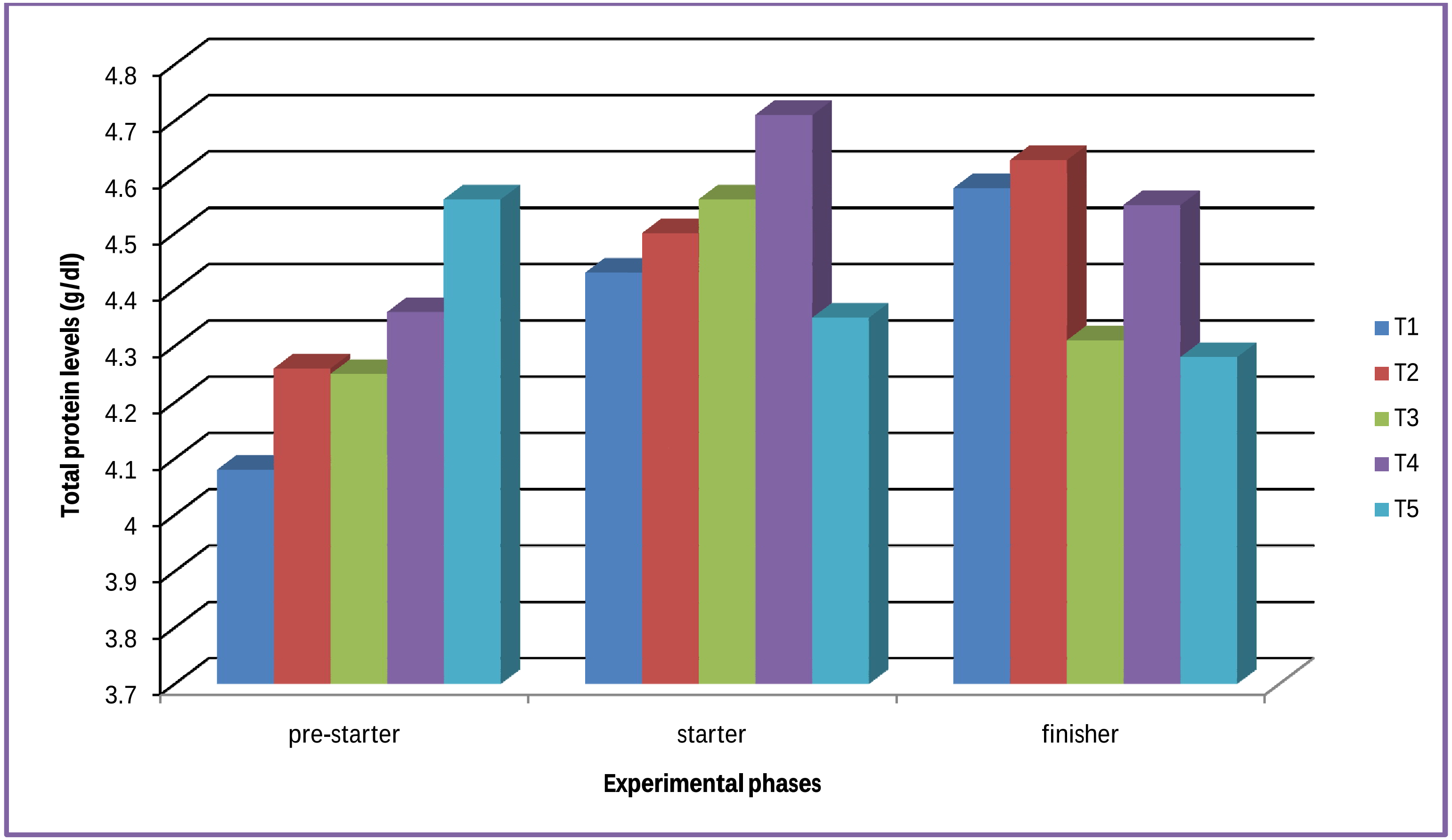


Fig.7 Effect of supplementation of shrimp waste meal on albumin (g/dl) of broilers

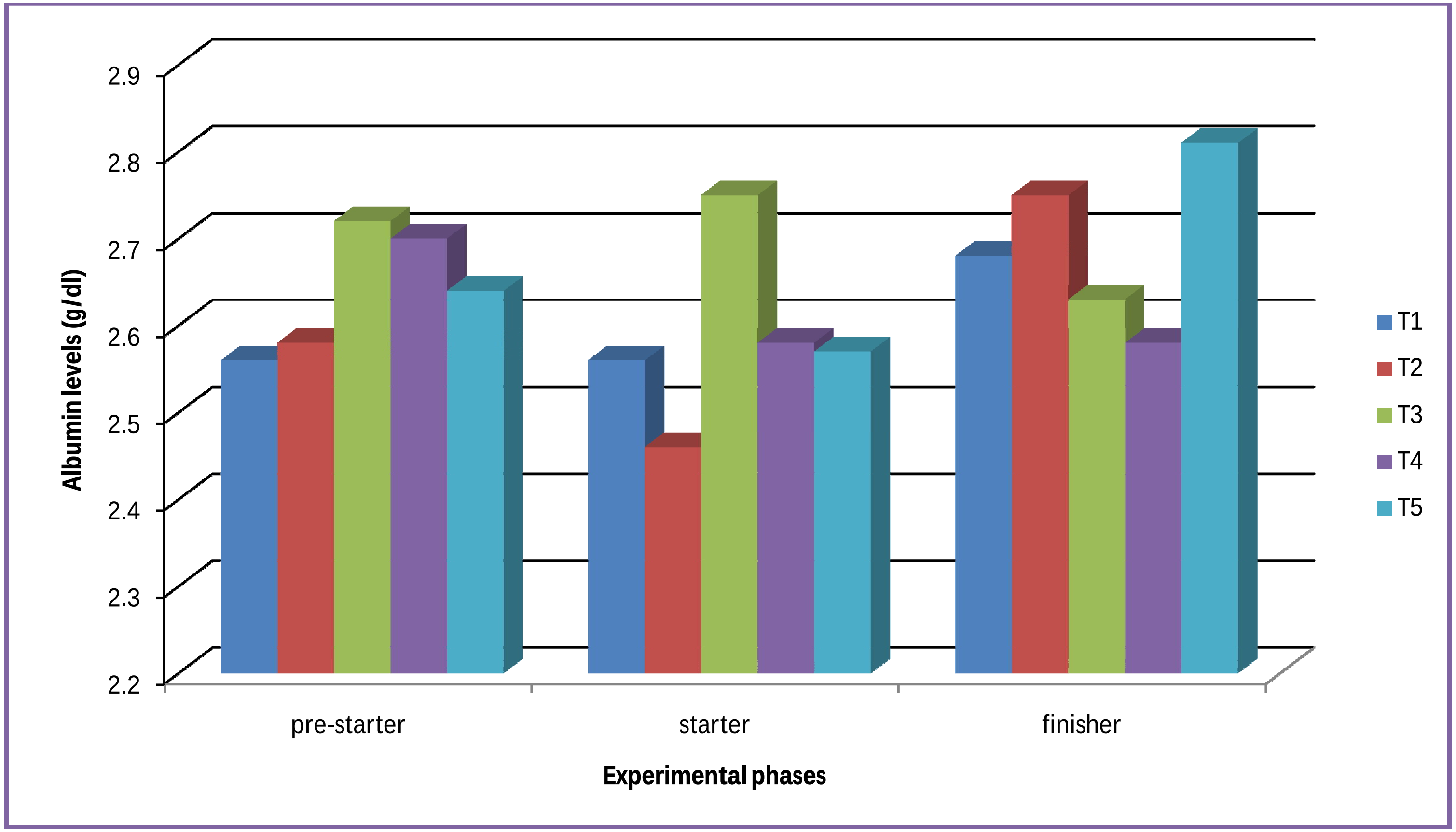
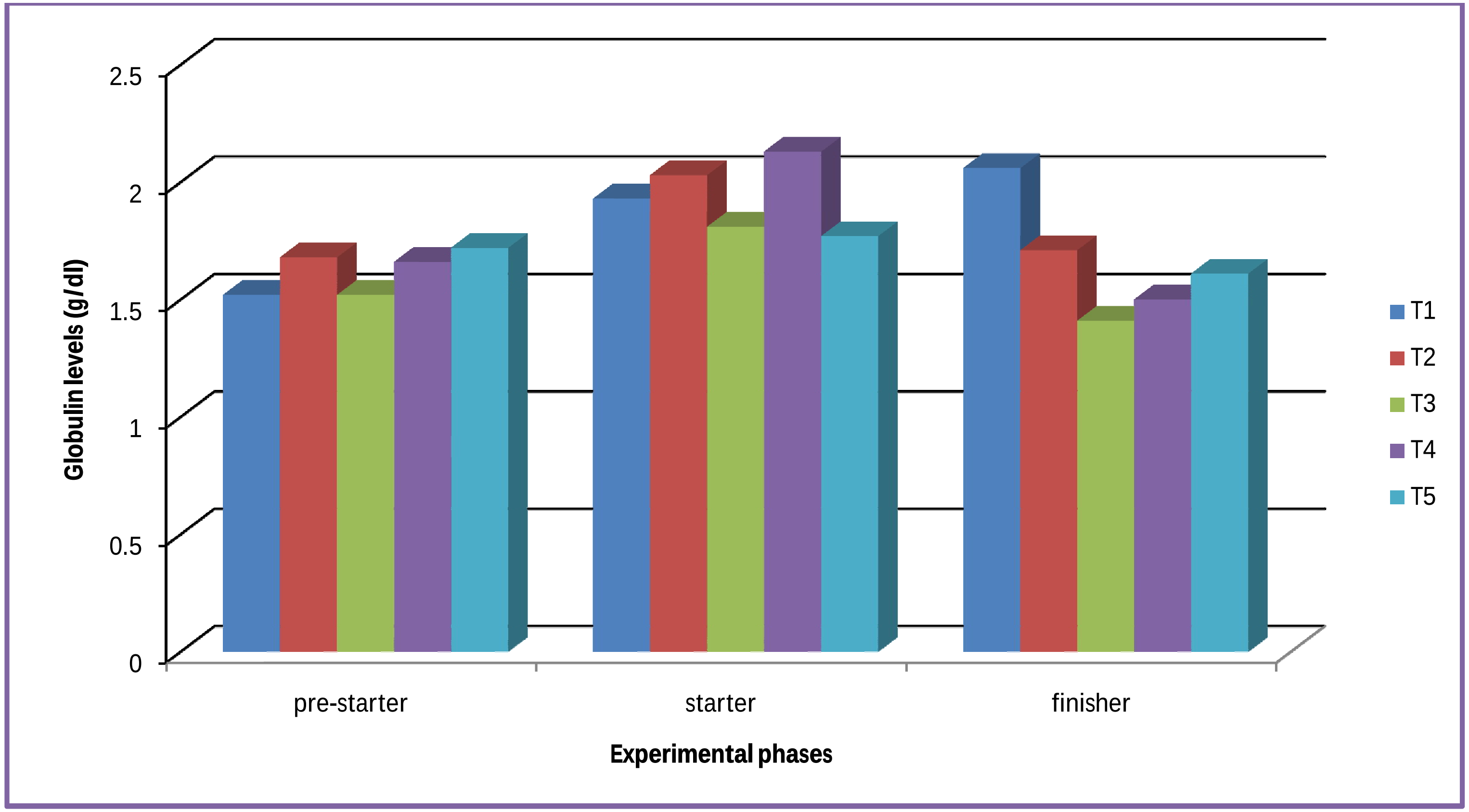


Fig.8 Effect of supplementation of shrimp waste meal on globulin (g/dl) of broilers



5.4.6.3 Serum cholesterol

During pre-starter and starter phases non significant differences were observed regarding serum cholesterol levels among different treatments (Table 14, 15 and 16, Fig: 9). A significant ($P<0.01$) reduction in the levels of cholesterol was noticed in the finisher phase with the increase in the level of SWM inclusion and the values ranged from 111.26 mg/dl (T_3) to 120.85 mg/dl (T_1). The observations of the present study are in harmony with the several earlier reports of Li *et al.,* (2007), Kobayashi and Itoh (1991), Razdan and Patterson (1994) and Razdan *et al*., (1997) who reported the beneficial effect of chito-oligosaccharides in reducing blood cholesterol levels. On contrary, Abiodun Adeyeye *et al.,* (2014) reported that the levels of serum cholesterol increased as the level of SWM substitution increased in turkey poults.

5.4.6.4 Serum glucose

During the experimental period, irrespective of the growth phase no significant differences were observed among different treatment groups regarding serum glucose levels (Table 14, 15 and 16 and Fig: 10). The results of present study were in conformity with the findings of Olayemi, (2001) who reported that there was no significant difference among dietary treatments.

Fig.9 Effect of supplementation of shrimp waste meal on cholesterol (mg/dl) of broilers

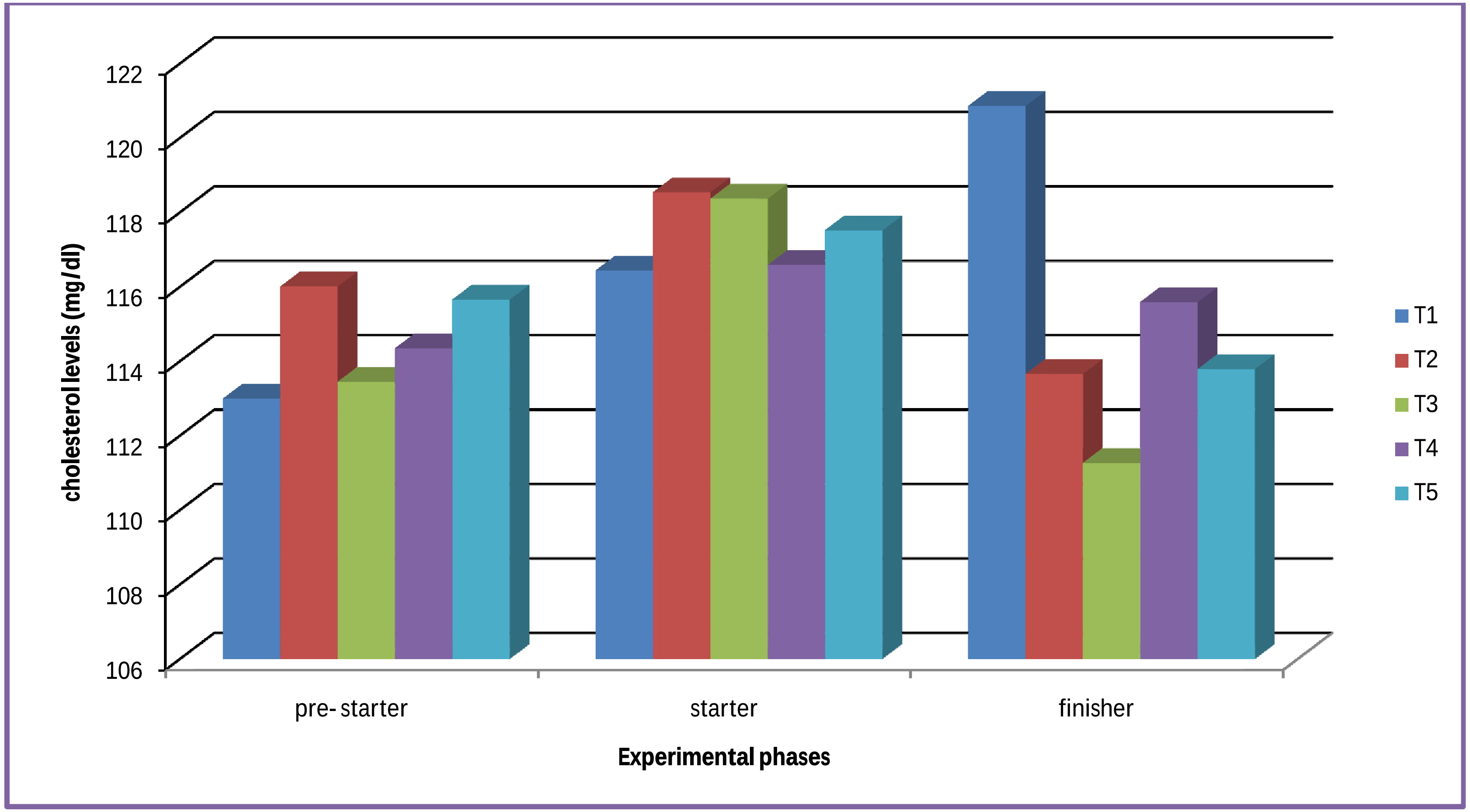
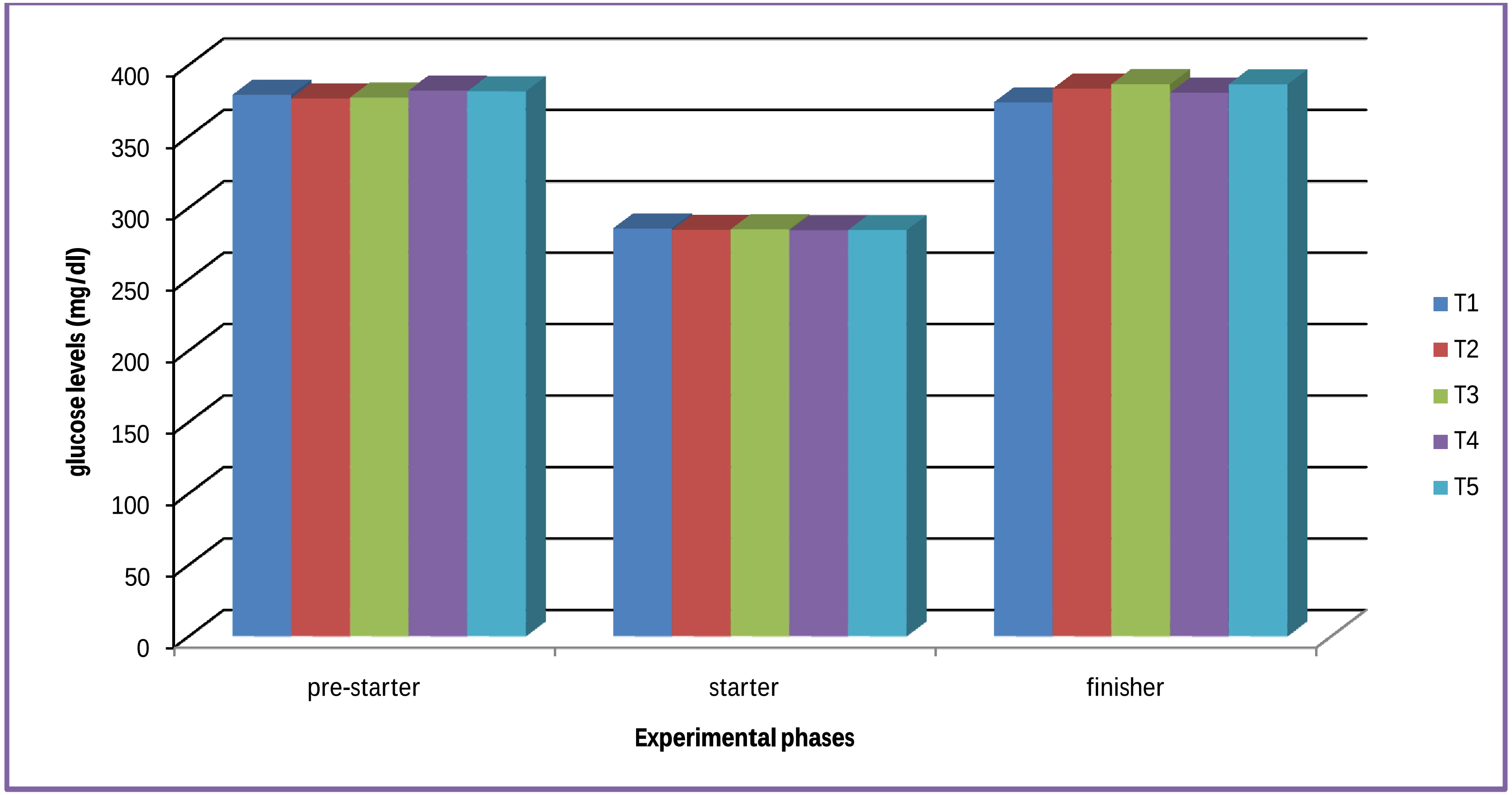


Fig.10 Effect of supplementation of shrimp waste meal on Glucose (mg/dl) of broilers



5.4.7 Blood cells

RBC count did not differ significantly among the treatments in all the phases of Experimental period. Significant ($P<0.01$) differences were observed in WBC count and the values were higher in the birds supplemented with SWM.

During pre-starter and starter phases higher ($P<0.01$) lymphocyte (%) was observed in birds fed T_2 and T_3 diets than in other treatments (Table 19). But, in finisher phase no significant difference was observed in lymphocyte (%) among treatments.

Increase in WBC (Meng *et al.,*2010), RBC (Zhou *et al.,*2009) were reported due to feeding of chito-oligosaccharide in poultry diets. Chen *et al*., (2009) reported that supplementation of 5g/kg chito-oligosaccharide did not affect the WBC, RBC count and lymphocyte (%).

5.4.8 Carcass characteristics

The live weight ($P<0.01$) at slaughter, hot carcass weight ($P<0.01$) and dressing (%) ($P<0.01$) were higher ($P<0.01$) in birds fed on T_4 and lower weight gains were observed with T_3 and T_5 diets. The better performance of the birds fed on T_4 diet might be due to improved utilization of SWM protein with the synthetic amino acids supplementation. On contrary, lower performance of the birds fed on T_3 and T_5 diets might be due to decreased utilization of nutrients at higher levels of chitin in the diets with the complete replacement of FM protein with SWM protein. Hector and Lourdes, (2005) and Fanimo *et al.* (1996) also reported decrease in live weight and carcass % with increase in SWM in the diet**.**

The liver, gizzard and heart weights at slaughter differed significantly ($P<0.01$) and higher weights were noticed in the birds fed on T_4 diets while, lower weight gains were observed with T_3 and T_5 diets. In contrary the findings of Okonkwo *et al*. (2012), Agunbiade *et al.* (2004), Olayemi, (2001) and Mahata *et al.* (2008) showed non significant effect of feeding different levels of shrimp waste on liver, gizzard and heart weights of broilers.

5.4.9 Cost economics

The feed cost/kg live weight gain (Fig: 11 & 12) was significantly lower ($P<0.01$) in T_4 fed birds and the highest ($P<0.01$) feed cost/kg live weight gain was observed in T_5 followed by $T_{3,}$ T_2 and by T_1 fed birds. The results of the present study revealed that the inclusion of SWM protein in T_3 and T_5 diets to replace 100% of fish meal protein with and without lysine and methionine supplementation could not match the birds fed on fish meal as indicated by the poor growth rate of the birds in the respective treatments.

The results of present study are in contrary with the findings of Okoye *et al.*, (2005) who reported that no significant difference was noticed among treatment groups.

5.4.10 Mortality

The percent mortality observed was 0, 2.66, 0, 1.33, and 1.33 in Pre-starter phase, 0, 2.66, 2.66, 5.33 and 4.0 in Starter phase and 2.66, 1.66, 1.33, 2.66 and 3.07 in Finisher phase in T_1 to T_5 fed birds, respectively. The cause for mortality of the birds was not due to SWM feeding and it was due to coccidiosis.

Fig:11 Effect of supplementation of shrimp waste meal on cost economics of broiler production

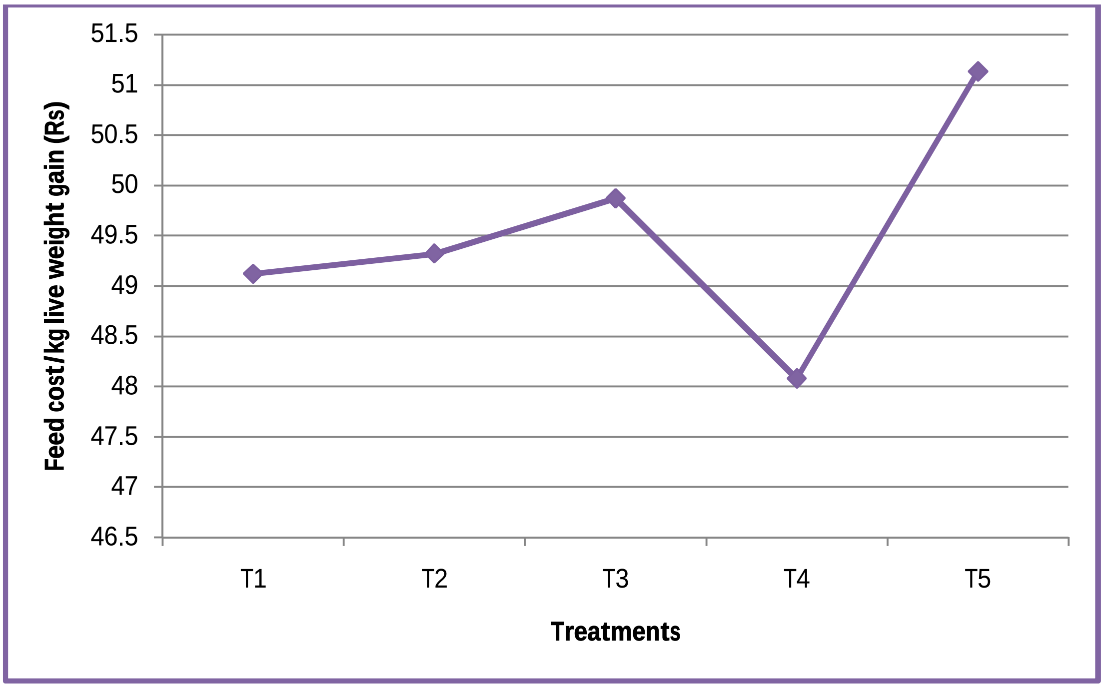
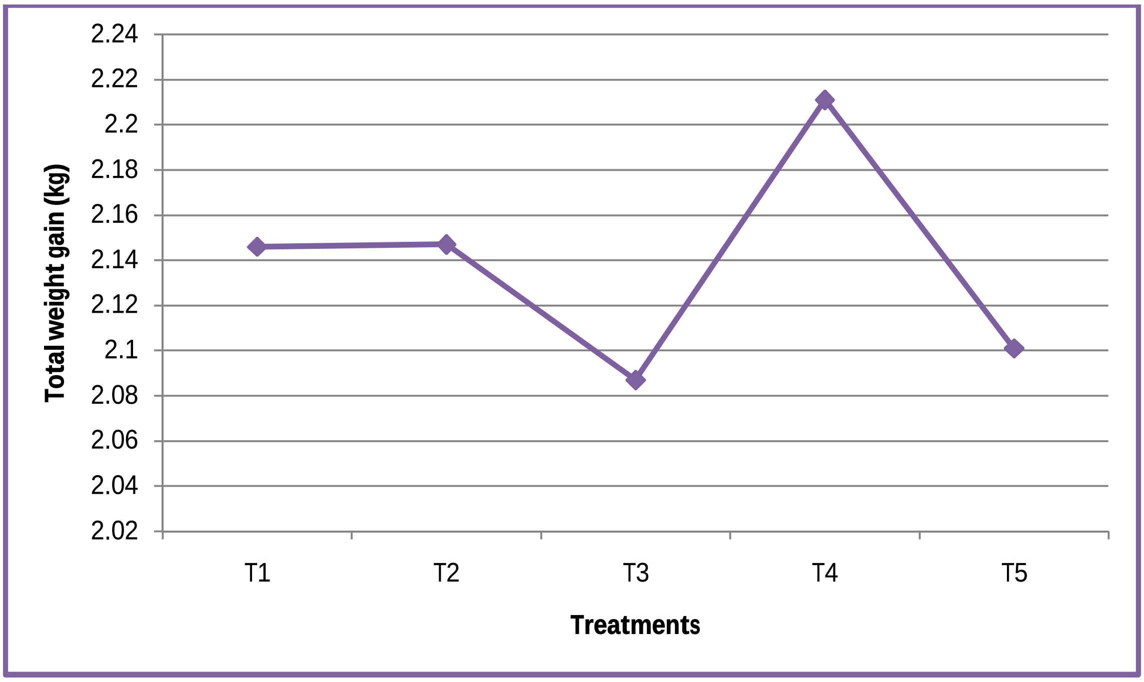


Fig:12 Effect of supplementation of shrimp waste meal on Total weight gain in broilers (kgs)



CHAPTER – VI

SUMMARY

Rapid urbanization along with huge demand for meat and eggs calls for increasing poultry production. Poultry Industry, with an average growth rate of 8-15 percent per annum, is one of the fastest growing segments of agriculture and allied sectors. Indian Poultry Industry ranks 3rd in egg production and 5th in broiler production (USDA, 2013). Poultry and poultry products at present command a major share of food of animal origin produced and consumed because of their cost effectiveness, easy availability, superior protein quality and wider acceptance by all sections of society irrespective of caste and religion.

Despite these advantages, most poultry farms have folded up because of the high cost of protein supplements and the incidence of disease. Research interest has therefore been awakened in the area of exploring alternative feed resources which have comparative nutritive value but are cheaper than the conventional protein sources. Shrimp waste meal is one of such unconventional protein sources of animal origin and it can be potentially channelled as a substitute for conventional protein sources in broiler diets to produce higher returns.

The present study was taken up by using five experimental diets to evaluate the effect of supplementation of locally available shrimp waste meal as a substitute to fish meal in broiler diets. Three hundred and seventy five day

old, straight run commercial vencobb male broiler chicks were distributed randomly to five treatments with three replicates of twenty five birds each. The experiment was carried out from day old to 42 days of age in three phases (pre-starter, starter and finisher).

Basal pre-starter, starter and finisher broiler diets were formulated with commonly available feed ingredients like maize, soybean meal, fish meal and de-oiled rice bran as per the Nutrient Requirements of Poultry, ICAR (2013). The CP (%) and ME (kcal/kg) contents of the basal pre-starter, starter and finisher diets were 22.04, 3000; 21.50, 3049 and 19.57, 3099, respectively.

In pre-starter phase five experimental diets were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 20% level (T_2) , 30% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. In starter and finisher phases five experimental diets were prepared by replacing fish meal protein of the basal diet with the shrimp waste meal protein at 50% level (T_2) , 100% level (T_3) and $T_{4,}$ T_5 diets were prepared by adding synthetic lysine and methionine to T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB, and 10 % fish meal. All diets were iso-nitrogenous and iso-caloric.

During the period of experimentation, weekly body weight gains and feed intake were recorded and feed efficiency was calculated accordingly. The results of present study revealed that there was no significant difference among treatment groups regarding body weight gain (BWG) during pre-starter phase.

But, in starter, finisher and overall growth phases birds fed with T_4 diet showed superior weight gain (P<0.01) compared to T_1 and T_2 whereas lowest weight gain was reported in the birds fed with T_3 and T_5. During the pre-starter and starter phases, the feed consumption differed significantly (P<0.01). Lower feed intake was observed in the groups fed with diets T_3 and T_5 (in which shrimp waste meal was used for the complete replacement of the fish meal). But, there was no significant difference in the feed intake among treatment groups during finisher and overall growth phases. During starter phase, feed efficiency differed significantly (P<0.01) among different treatments. No significant difference was noticed among treatments in pre starter, finisher and overall growth phases. In all the phases better feed efficiency was observed in the group fed with the diet T_4 (containing 50% fish meal protein replaced with shrimp waste meal protein along with the synthetic amino acids supplementation).

Two birds per replicate and a total of six birds per treatment were selected randomly at the end of four weeks and at the end of experiment to carry out metabolism trails. No significant differences were noticed regarding the digestibility of DM and CF among treatments in starter and finisher phases. However, in starter and finisher phases the digestibility of CP and EE was significantly (P<0.01) higher in birds fed T_4 ration and lower values were observed in those fed with T_3 and T_5 diets.

The *E.coli* and *Salmonella* count (log cfu/g) of cecal contents were studied in two birds per replicate and a total of six birds per treatment at the end of two, four and six weeks were sacrificed. During entire experimental period irrespective of the growth phase with the increase of SWM in the diet there was a significant ($P<0.01$) reduction in the *E.coli* count (log cfu/g) and *Salmonella* count (log cfu/g) of cecal contents in broilers.

A slaughter was performed at the end of pre-starter, starter and finisher phases and blood samples were collected from two birds per replicate thus, a total of six birds per treatment were slaughtered for estimation of serum biochemical parameters (total protein, albumin, globulins, glucose and cholesterol). No significant differences were observed in serum total protein among treatment groups during pre-starter, starter and finisher phases but, there was a slight increase in the serum total protein content in the groups fed with SWM diets when compared to control. No significant difference was noticed regarding serum albumin and globulin values among treatment groups during pre-starter, starter and finisher phases. During pre-starter and starter phases non significant differences were observed regarding serum cholesterol levels among different treatments. A significant reduction in the levels of cholesterol was noticed in the birds fed with T_2, T_3, T_4 and T_5 when compared to control during finisher phase. During the experimental period, irrespective of the growth phase no significant differences were observed among different treatment groups regarding serum glucose levels.

During pre-starter, starter and finisher phases hematological parameters (RBC, WBC and lymphocyte %) were estimated. RBC count did not differ significantly among the treatments in all the phases of experimental period.

Significant ($P<0.01$) differences were observed in WBC count and the values were higher in the birds supplemented with SWM. During pre-starter and starter phases higher ($P<0.01$) lymphocyte (%) was observed in birds fed T_2 and T_3 diets than in other treatments. But, in finisher phase no significant difference was noticed in lymphocyte (%) among treatments.

The live weight, hot carcass weight and the dressing (%) were found significantly higher ($P<0.01$) in birds fed T_4 diet when compared to birds fed other diets. The liver, gizzard and heart weights at slaughter differed significantly ($P<0.01$) and higher weights were noticed in the birds fed on T_4 diets while, lower weight gains were observed with T_3 and T_5 diets .

Based on the results obtained in the present study the following conclusions can be drawn:

1. Substitution of FM protein with SWM protein along with the supplementation of synthetic amino acids resulted in better body weight gains, feed efficiency and cost economics.
2. Supplementation of synthetic amino acids (lysine, Methionine) was found to be beneficial in improving the utilization of SWM protein in broiler diets.
3. A significant reduction in the gut pathogen population (*E.coli, salmonella*) irrespective of the growth phase was observed with the SWM supplementation because of the prebiotic effect of chitin present in it.

4. Cholesterol content of the serum was reduced in the birds supplemented with SWM and it appears to be beneficial from the human health point of view.
5. So, it can be concluded that the shrimp waste meal can be utilized as a promising alternative protein source to fish meal in broiler diets.
6. Fish meal Protein can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets along with amino acids supplementation for good economic returns and productive performance.

CHAPTER - VII

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