STUDIES ON THE NUTRITIVE VALUE AND UTILIZATION OF SQUILLA (*Oratosquilla nepa*) MEAL IN POULTRY RATIONS

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DECLARATION

I, **Mr.V.RAVINDER REDDY,** hereby declare that the thesis entitled **STUDIES ON THE NUTRITIVE VALUE AND UTILIZATION OF SQUILLA (*Oratosquilla nepa*) MEAL IN POULTRY RATIONS** submitted to the Acharya N.G. Ranga Agricultural University for the degree of **DOCTOR OF PHILOSOPHY** in the faculty of Veterinary Science is a result of the original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Date: 6th December, 1996 (**Mr.V.RAVINDER REDDY**)

Place: Hyderabad.

CERTIFICATE

Mr. V.RAVINDER REDDY, has satisfactorily prosecuted the course of research and that the thesis entitled STUDIES ON THE NUTRITIVE VALUE AND UTILIZATION OF SQUILLA (*Oratosquilla nepa*) MEAL IN POULTRY RATIONS submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for the degree of any university.

Date: 6, December, 1996.
Place: Hyderabad.

(DR.V.RAVINDRA REDDY)
MAJOR ADVISOR

CERTIFICATE

This is to certify that the thesis entitled **STUDIES ON THE NUTRITIVE VALUE AND UTILIZATION OF SQUILLA (*Oratosquilla nepa*) MEAL IN POULTRY RATIONS** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the faculty of Veterinary Science of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Dr.V. RAVINDER REDDY** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the 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 the investigations have been duly acknowledged by author of the thesis.

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(V.RAVINDER REDDY)

LIST OF ABBREVIATIONS AND USE

AME	:	Apparent Metobolizable Energy
C	:	Centigrade
Ca	:	Calcium
cm	:	centimeter
cm^2	:	Square centimeter
Cr_2O_3	:	Chromic oxide
d	:	day
df	:	degrees of freedom
DMB	:	dry matter basis
DNP lysine	:	dinitro-phenyl lysine
FE	:	feed efficiency
g	:	gram(s)
GPV	:	gross protein value
h	:	hour(s)
HCl	:	hydrochloric acid
ICU	:	International Chick Unit
IU	:	International Unit
kg	:	kilogram(s)
mcg	:	microgram
Me	:	Metabolizable energy
ME_n	:	nitrogen corrected metabolizable energy
mg	:	milli gram
MJ	:	mega joules
ml	:	milli litre
mm	:	milli meter

MSS	:	Mean Sum of Squares
mt	:	minute
mμ	:	milli microns
N	:	nitrogen
NFE	:	nitrogen-free- extract
nm	:	nanometer
PDAB	:	para-dimethyl-amino-benzaldehyde
ppm	:	parts per million
ppb	:	parts per billion
Rs.	:	rupees
v/v	:	volume/volume
wk	:	week(s)
WL	:	White Leghorn
μg	:	microgram
%	:	per cent
@	:	at the rate of

C O N T E N T S

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LIST OF ILLUSTRATIONS

Name of the author	:	**V. RAVINDER REDDY**
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ABSTRACT

Studies were conducted on evaluation of nutritive value and utilization of squilla (*Oratosquilla nepa*) meal *Vis-a-vis* fish meal protein for broilers and WL layers.

The crude protein content of squilla meal was 339.7 g/kg while that of fish meal was 546.0 g/kg. It had higher crude fibre and total ash contents (139.2 and 383.1 g/kg) than fish meal (32.0 and 314.5 g/kg). Its trace mineral content was higher, while the amino acid profile was lower. It was low both in GPV (68%) and protein digestibility (66%) than fish (72 and 74% respectively). Its metabolizable energy content was 7.13 MJ/kg as against 8.08 MJ/kg in fish meal.

Series of biological trials were conducted using graded levels (41.7, 83.4, 125.1 and 166.8 g/kg) of raw squilla meal in broilers (Experiment 1) and at 44.5, 88.9 and 133.4 g/kg levels in WL layers (Experiment 3) to suggest the optimum levels of inclusion in their respective diets replacing fish protein. To explore the possibilities of using squilla meal as a sole source of animal protein, the fish protein was replaced by 50, 75 and 100 per cent of raw and autoclaved

squilla meal and raw squilla meal diets supplemented with 0.5, 1.0 and 2.0 g/kg proteases enzyme premix and its utilization in broilers was studied (Experiment 2). In layer diets, the test materials were used at 88.9 and 133.4 g/kg levels (Experiment 4).

The criteria of evaluation of squilla meal were based on growth, feed to gain ratio, weight of visceral organs and lengths of different segments of intestines; egg production, feed efficiency and egg quality parameters in WL layers.

In experiment 1 (1-42 d), the body weight gains were similar with reference and with varying dietary levels of squilla meal, except a numerically lower weight gains on higher level (166.8 g/kg) of squilla meal. The feed intake was not significantly influenced by different dietary levels of squilla meal. Feed efficiency was lower on higher levels of squilla meal. The feed cost per kg live weight gain with all levels of inclusion of squilla meal was however comparable. The lengths of duodemum and caecum increased linearly ($P=0.0435$; $P=0.0028$ respectively) with the increasing levels of squilla in diet. The visceral organs weight did not show any specific pattern.

The raw squilla meal at higher level (166.8 g/kg) significantly ($P<0.01$) lowered weight gains. Autoclaving failed to improve the performance in broilers (8-42 d, experiment 2). Addition of bacterial proteases enzyme at 0.5 and 1 g/kg levels to the highest (166.8 g/kg) squilla meal diets, improved body weight gains, feed efficiency and decreased feed consumption compared to raw squilla meal diets, but the values were comparable with control.

The hen-day egg production (30-45 wk) upto 88.9 g/kg level was comparable to control (experiment 3). However, at 133.4 g/kg replacement level it was significantly ($P<0.01$) lowered. The feed consumption was unaffected, while the feed efficiency was comparable at lower level but significantly ($P<0.05$) poorer to control at higher levels (88.9 and 133.4 g/kg). Egg quality parameters were influenced significantly, but did not show any specific trend.

In the diets with both raw and autoclaved squilla meal at 88.9 g/kg level in WL layer (25-40 wk, experiment 4) the hen-day egg production was comparable with control. However, at 133.4 g/kg level it was significantly ($P<0.01$) lowered. The feed efficiency on autoclaved squilla meal diets was significantly ($P<0.05$) poorer to control. Addition of bacterial proteases (0.5 g/kg) to raw squilla meal diets improved the hen day egg production and feed efficiency in comparison to their respective raw squilla meal diets but comparable to control.

It is inferred from the above results that squilla meal can safely replace $3/4^{th}$ and $2/3^{rds}$ of fish protein in broiler and layer diets respectively. However, enzyme supplementation, at 0.5 g/kg diet, appears to be essential for complete replacement of fish protein for both broilers and layers.

INTRODUCTION

CHAPTER - I

INTRODUCTION

The phenomenal growth of poultry farming during the last three decades, has given it the status of one of the top 10 industries in India. Still the profitability of poultry unit is largely dependent upon the feed cost, which is a major input component in poultry production. Fish meal is most commonly used (8-10 %) animal protein source in poultry rations. The cost of fish meal is increasing, even though its production has registered a menifold increase year after year, this increase is not commersurate with the requirements of fast growing poultry industry. At this juncture, using alternate animal protein sources will drastically reduce dependency on fish meal.

Nutritionists succeeded in their efforts in using several alternate unconventional animal protein sources in poultry feeds, like silkworm pupae meal, meat meal, blood meal, liver meal, hatchery by-product meal, feather meal etc. But due to the limiting factors like contamination, low protein digestibility, problems in processing etc., their use in poultry feeds has been limited. Moreover, restricted and inconsistent availability, besides lack of quality control could not stop dependancy on fish meal. Squilla meal may play a promising role as an animal protein source replacing fish meal in poultry feeds.

Squilla (*Oratosquilla nepa*), a stomatopod crustacean is abundantly available along Indian coast besides China, Thailand, Malaysia, Philippines etc. These are trapped in large quantities (one lakh tonnes per year) in India along with prawns during trawling operations. Being an animal origin protein, not consumed by human beings is widely used in fish meal plants for the production of fish meal. Now, these are available in market as a whole meal and can be diverted for feeding livestock and poultry.

Squilla meal is known by different names in different parts of the world. Squilla, Mantis shrimp, Latreille, (English); Keeda, (Hindi), Teluroyya, Purugu (Telugu); Bombay jinga, Chelly, Pucha, Puchee and Cadal poochi in other vernacular languages.

The price of squilla meal most of the times is substantially lower than that of both whole fish and fish meal at the trapping sites and also in marketing centres. Information available on its nutritive value and level of inclusion in poultry rations is very limited. Its incorporation at the expense of fish meal in poultry rations would certainly reduce cost of poultry ration, besides reducing the dependency on contaminated and or adulterated inferior quality fish. A series of experiments were thus felt necessary to evaluate the nutritive value of squilla meal and its feasibility as source of animal protein in poultry rations, with the following objectives.

1. To evaluate the nutritive value of squilla meal for poultry by *in vitro* and *in vivo* studies.

2. To study the effect of feeding squilla meal on the performance of broilers.

3. To study the effect of feeding squilla meal on egg production, feed efficiency and egg quality characteristics in layers.

4. To improve the nutritive value and to increase its inclusion level by subjecting squilla meal to autoclaving and addition of enzyme premix.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

In this chapter, the relevant literature on the nomenclature, habitat and morphology, availability, chemical composition and nutritive value of squilla meal has been reviewed. The literature on utilization of squilla meal is scanty; hence the utility of other related marine products for poultry have also been reviewed.

2.1 NOMENCLATURE

Squilla (*Oratosquilla nepa*) is known by the local names 'chelly', 'puchee', 'puchi', 'keeda', teluroyya, purugu etc. It's classification has been detailed below (Jordan and Verma, 1976)

Phylum	:	Arthropoda
Class	:	Crustacea
Sub-class	:	Malacostraca
Division	:	Hoplocarida
Order	:	Stomatopoda
Family	:	Mantis shrimps
Genus	:	Squilla
Species	:	*Oratosquilla nepa*

2.2 HABITAT AND MORPHOLOGY

Squilla are prawn like marine crustacean, living in burrows of the sand or mud at the bottom of the sea.

The catches of stomatopods have been exceptionally high in Karnataka state particularly along the south Kanara coast. Around 50 per cent of India stomatopods catch is obtained from Karnataka state alone (Sukumaran, 1988). The catches of *Oratosquilla nepa* along the south Kanara coast were maximum during 1981-82, amounting to 2543.3 tonnes at a catch rate of 16.5 kg/h at Mangalore and about 1868.8 tonnes at 16.7 kg/h at Malpe coast respectively. The catch was high during January-April months (Sukumaran, 1987).

Stomatopods, being not consumed by human beings, due to their lesser flesh content and presence of large number of spines on the body (Sukumaran, 1988) these were earlier thrown back into sea. However, due to scarcity of animal protein sources, oflate, it has been found that it is a good raw material for converting into fish meal and poultry feed hence there is a lot of demand for the same by the fish meal plants. It also fetches a reasonable price in recent years. The stomatopods are mostly sundried in the harbour premises and sold to fish meal plants (Sukumaran, 1988). Hence, commercial fish meal invariably contain certain amount of squilla meal in them. Whole squilla meal are also available for livestock and poultry feeding.

The body of squilla (Fig.1) is elongated. The mature females measure in the range of 83 mm to 108 mm with an average of 95 mm. Its body is divisible into head, thorax and broad abdomen. Squilla is active and predatory. It catches hold of the prey with the powerful maxillipedes (Jordan and Verma, 1976).

The species appear to grow to a length of about 108 mm by the end of first year and survives for 15-16 months. The size at maturity is estimated at 95 mm. Females generally outnumbered males. Chi-square test showed significant variation from 1:1 ratio in the distribution of sexes (Sukumaran, 1987).

2.3 AVAILABILITY

Among the crustaceans exploited by commercial nets, perhaps, stomatopods are economically the least important. The stomatopod fishery is exclusively supported by a single species namely *Oratosquilla nepa* all along the west coast in India(Sukumaran, 1988).

Squilla is a major fishery waste along with other crustaceans in countries like USA, China, Thailand, Malaysia, Philippines, South Africa and Mexico (Ramachandra Nair *et al.*, 1987). In India, they are abundantly available along the west coast. These are trapped in large quantities along with prawns during trawling operations (Govindan, 1984); about one lakh tonnes per year (Ranjhan, 1993).

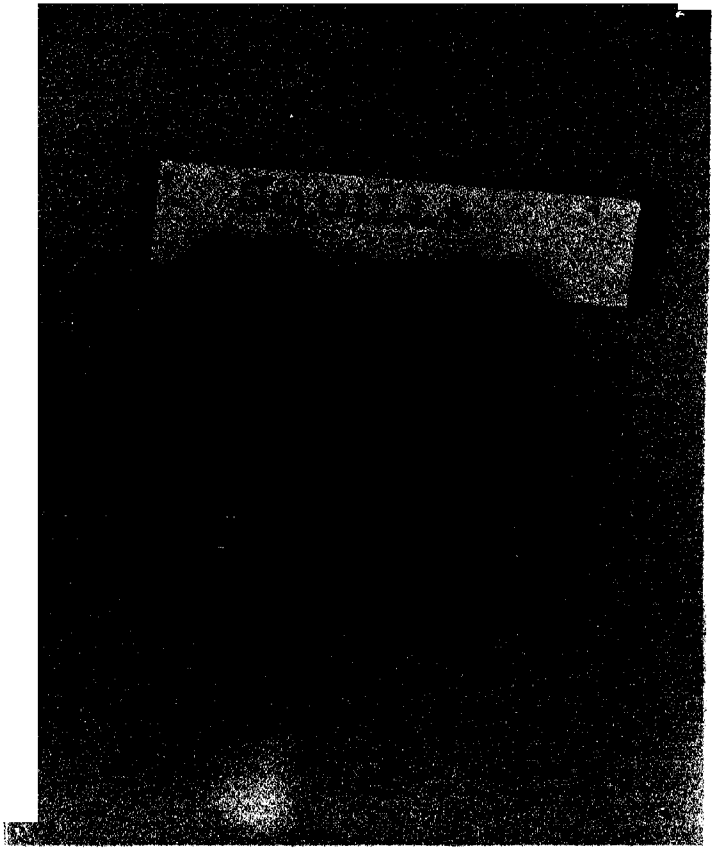


Fig. 1 Dorsal view of Squilla (*Oratosquilla nepa*)

2.4 CHEMICAL COMPOSITION

The literature on the chemical composition of squilla meal was limited and it was found to vary greatly depending upon the stage of maturity of squilla and type of drying process adopted. The reported data pertains to composition of both raw samples of squilla and its protein extract.

2.4.1 Proximate composition

The proximate composition of squilla reported (Table 1) by Madhavan and Ramachandra Nair (1975) revealed that it contained 447.1 g crude protein, 345.2 g ash and 26.8 g fat per kg. They have also demonstrated marginal differences in chemical composition between squilla and prawn waste (397.6 g crude protein, 311.3 g ash and 50.5 g per kg fat). Similarly Govindan (1984) also indicated that the chemical composition of squilla is very much alike to that of prawn shell waste.

Analysing the protein extract prepared from squilla, Mathew *et al.* (1982) reported high crude protein (655 g per kg) and moderate levels of ash (136 g per kg) and fat (79 g per kg). Lekshmy Nair *et al.* (1991) prepared the squilla protein powder from aquous exudate obtained from meat bone seperator and the extract was reported to contain 943 g dry matter, 641 g protein, 25 g fat and 127 g per kg ash.

Table 1: Proximate composition of squilla (g/kg on DMB) as reported in the literature

	Squilla[1]	Protein extract squilla[2]	Squilla protein powder[3]
Moisture	--	53.0	51.0-62.0
Crude protein	447.1	692.0	602.0-680.0
Ether extract	26.8	83.0	22.0-28.0
Chitin	147.0	--	--
Total ash	345.2	144.0	118.0-135.0

1. Madhavan and Ramachandra Nair (1975)
2. Mathew *et al.* (1982)
3. Lekshmy Nair *et al.* (1991)

Table 2: Essential amino acid pattern of squilla protein to FAO/WHO amino acid scoring pattern (g/16 g N, on DMB) as reported in the literature.

Amino acid	Squilla protein	FAO/WHO pattern
Isoleucine	5.2	4.0
Leucine	7.0	7.0
Lysine	7.1	5.5
Methionine + cystine	3.6	3.5
Phenylalanine + tyrosine	7.3	6.0
Threonine	3.2	4.0
Tryptophan	0.8	4.0
Valine	5.0	5.0

Mathew *et al.* (1982)

2.4.2 Amino acid profile

The amino acid profile of squilla protein extract revealed to contain all essential amino acids (Table 2) in adequate amounts, 5.2 g isoleucine, 7.0 g leucine, 7.1 g lysine, 3.6 g methionine + cytine, 7.3 g phenylalanine + tyrosine and 5.0 g per 16 g N of valine. However tryptophan, 0.8 g per 16 g N and threonine 3.2 g per 16 g N, were comparatively lower (Mathew *et al.*, 1982).

On comparison of the amino acid pattern of squilla protein extract to the provisional pattern set by FAO/WHO (FAO/WHO, 1973) lysine, isoleucine, valine and the sulphur containing amino acids were shown to be adequate but threonine and tryptophan were limiting. The overall amino acid score was however equivalent to 80 per cent of FAO/WHO pattern. The lysine content of squilla protein extract is much higher than that contained in the pattern and is almost equal to that of casein and casein + squilla protein combination (Mathew *et al.*, 1982).

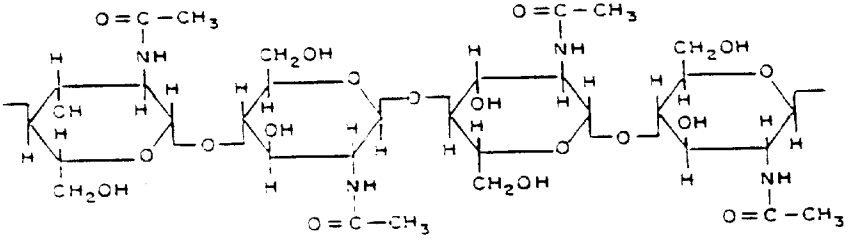
2.4.3 Chitin content

The exoskeleton of squilla contains chitin and it provides skeletal support and an armour against attack by other marine animals. The squilla contained 147 g/kg of chitin (Madhavan and Ramchandra Nair, 1975).

2.4.3.1 Structure of chitin and chitosan

Chitin is a macro-molecular, linear polymer of anhydro-N-acetyle-D-glucosamine (N-acetyle-2-amino-2-deoxy-D-glucose). Its molecular structure is shown in Fig.2. It is insoluble in water and most organic solvents. Chitosan is an N-deacetylated product of chitin. Chitin and chitosan are not deleterious to poultry (Arai *et al.*, 1968).

CHITIN



Molecular structure of chitin

2.4.3.2 Estimation of chitin

The molecular structure of chitin is similar to that of cellulose, the chitin molecule differing only in substitution of acetylamide for the hydroxyl group on corbon-2 of the glucose units (Richards, 1953). Therefore, one might anticipate that chitin may be a fibrous component of crustacians as cellulose is in feeds of plant origin.

After comparing Welinder and Van Soest acid detergent fibre methods, it was concluded that chitin is a fibrous component of shell fish meals, and the Van Soest acid detergent fibre method is acceptable for determination of shell fish chitin (Stelmock *et al.*, 1985).

2.4.3.3 Utilization of chitin for poultry

Ramachandran Nair *et al.* (1987) have shown that by using chitin at 0.5 per cent in broiler diets, significantly increased body weight gain. It was attributed that chitin, might have acted as a growth promoter in broiler chicken.

Nutritional studies with broiler chicken have shown that microcrystalline chitin as diet supplement upto 20 per cent controlled diarrhoea commonly occurring when whey is added to the chicken diet (Austin *et al.*, 1981). These findings were further strengthened by the work of Zikakis *et al.* (1982) who have reported that chitin improved the digestion of whey in the broiler chicks.

Supplementation of chicken diets with chitin stimulated the growth of *Bifidobacteria* that can synthesize lactase. Lactase has been the limiting factor for successful utilization of whey and other milk products containing lactose, as chicken feed supplement (Spreen *et al.*, 1984).

Hirano *et al.* (1990) conducted studies on digestibility of chitin and chitosan in hens. Supplementation of chitin at 2 per cent and chitosan at 5 per cent in layer ration resulted in 92 per cent and 98 per cent digestibility, respectively. They also observed that no abnormal symptoms were noticed in hen/broilers by feeding < 1.4 g of chitosan/kg of body weight per day. But, excessive amount of chitosan (3.6-4.2 g/kg body weight per day) in hens decreased appetite and egg laying rate, due to incomplete digestion of chitosan.

Narahari *et al.* (1991) have revealed that dietary inclusion of chitin at 0.2, 0.4 and 0.6 per cent levels in broiler diets, did not influence significantly ($P>0.05$) the weight gains. On supplementation of chitin in diets, broiler diets at 0.6 per cent gave body weight gains (660 g) at 6 weeks age against control (643 g). But, the feed consumption showed gradual reduction resulting in a significant ($P<0.01$) improvement in feed efficiency (3.16) in favour of high chitin group than control (3.86).

Kobayashi and Itoh (1991) studied the effect of dietary chitin and chitosan on growth and abdominal fat deposition in chicken. Addition of 5 per cent chitin or chitosan in the diets did not affect body weight gain and feed efficiency. In high fat diet, addition of 5 per cent chitosan decreased the apparent fat absorption and abdominal fat pad weight in chicken.

Ramachandran Nair *et al.* (1993) studied the effect of feeding chitin in poultry. Chitin at 0.5 per cent level in broilers showed significant increase in weight gain and better feed conversion ratio. However, in White Leghorn layers a drop in egg production was observed.

2.4.4 Protein quality of squilla meal

The protein extract prepared from squilla was evaluated for its nutritional quality in comparison to casein for growth rate and protein efficiency ratio (PER) in rats. Weight gains on squilla protein was found to be 58 g as against 65 g and 66 g obtained on casein alone and casein + squilla protein (1:1) respectively. Similarly the PER value for squilla was 2.83 in comparison to 2.92 and 2.86 recorded with the other two groups. The lower value for squilla protein was basically due to lowered feed intake (205 g) with a resultant lowered (20.5 g) protein intake (Mathew *et al.*, 1982).

Nutritional studies on the evaluation of squilla protein in rats indicated that the squilla protein powder has average protein quality. Its ingestion at the proper level (20% of protein source in diet) promotes satisfactory growth (25.8 g vs 25.0 g on test protein vs casein protein) in rats at 3 weeks of age (Lekshmy Nair *et al.*, 1991).

2.5 UTILIZATION OF SQUILLA MEAL AS A FEED INGREDIENT

There is no literature available on utilization of squilla meal in poultry but few research articles have been published on utilization of squilla in carps and rats.

Nandeesha *et al.* (1989) studied the influence of squilla meal-based diets on the growth and organoleptic quality of common carp. It was observed that feeding of dried squilla powder plus soyabean meal and saridin oil improved growth (73.67 g) against soyabean meal plus fish meal diet at 14 weeks of age. Digestibility, feed conversion and organoleptic quality of carp were also improved. Squilla meal was considered as suitable feed supplement for carp.

The studies on the evaluation of squilla protein at 10 per cent in rat diet revealed that the growth and reproductive performance were comparable with casein based diets. No adverse effect on appearance, behaviour and survival of rats on experimental diets were observed (Lekshmy Nair *et al.*, 1991).

2.6 UTILIZATION OF OTHER RELATED MARINE PRODUCTS

Parkhurst *et al.* (1944) conducted biological trial on laying pullets by feeding crab meal or fish meal on an equal protein basis and with the mineral content. No differences were observed on egg production, efficiency of feed utilization, fertility, hatchability, egg weight, yolk color, albumin quality and shell texture.

Potter and Shelton (1973) indicated that 3 or 6 per cent crab meal in turkey poult diets increased body weight significantly ($P<0.05$) by 5.2 per cent and 6.8 per cent during first 4 weeks of age. However during 5-8 week the improvement in growth was insignificant (1.4 and 1.3%) respectively on the 3 per cent and 6 per cent crab meal. Feed efficiency was unaffected.

Ilian *et al.* (1985) have evaluated shrimp by-catch meal for poultry. Its supplementation at 5 per cent in broiler diet significantly enhanced body weight gains. It was concluded that though shrimp by-catch meal was inferior to menhanden fish meal in sulphur amino acid and crude protein content, its higher calcium and phosphorus content might be a factor in growth stimulation.

Jarvuin *et al.* (1972) revealed that supplementation of shrimp by-product meal replacing fish meal at equal protein levels in diets of growing chickens, gave a somewhat

slower growth. However, on supplementation with lysine improved body weight gains.

Shrimp shell powder (SSP) as part of substitute for dry fish in the ration of broiler chickens were tried. The SSP had 32 per cent crude protein. The body weight gains of the chicks during 8 weeks was less on the replacement of SSP at 5 or 10 per cent. However, at 6 weeks the weights were similar to control on 5 per cent SSP replacement. Although the biological efficiency was better in the controls than in those given 5 per cent SSP (Menachery *et al.*, 1978).

Tolokonnikov *et al.* (1984) evaluated the shrimp meal for broilers. The shrimp meal contained an average crude protein of 59, fat 9, ash 11 and chitin content of 6 per cent. The shrimp meal replacement upto 25 per cent of protein content of control diet had no effect on serum protein and Ca concentration. However shrimp meal at more than 10 per cent dietary protein decreased growth performance and carcass yield.

Crab meal, a dried waste of the industry is an animal protein concentrate of high mineral content. The commercial meal contained 32.7 per cent of crude protein, 12.9 per cent of crude fibre (mainly chitin) and 41.6 per cent of ash. It was a good source of Mn and Fe (Lubitz *et al.*, 1943)

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

Investigations were carried out in the Department of Poultry Science, College of Veterinary Science, Rajendranagar to evaluate the nutritive value and utilization of squilla meal for poultry. The materials and methods employed to determine the nutritive value of the squilla meal and detailed in Part I, while those employed to study on its utilization are given in Part II. The detailed experimental plan of these investigations is given in Table 3.

PART - I

3.1 NUTRITIVE VALUE OF SQUILLA MEAL AND FISH MEAL

The bulk quantities of squilla meal and fish meal materials used in these studies were obtained from local market to evaluate their nutritive value.

3.1.1 *In vitro* studies

Representative samples of squilla meal and fish meal were analysed for proximate principles, chitin content and for aflatoxin presence. Amino acid composition, available lysine and mineral content were also determined. Analysis for all constituents were made on duplicate samples.

Table 3: Experimental plan on the investigations on nutritve value and utilization of squilla meal for poultry

Nutritive value of squilla meal		Feeding trials on utilization of squilla meal in chicken
In vitro studies	In vivo studies	
1. Proximate and mineral composition	1. Gross protein value	1. Utilization of squilla meal for growth
	2. Protein digestibility	(a) Raw squilla meal in broilers (Experiment 1)
2. Aflatoxin content	3. Metabolizable energy	(b) Raw and autoclaved squilla meal and raw squilla supplemented with enzyme in broilers (Experiment 2)
3. Amino acid profile		
4. Available lysine		2. Utilization of squilla meal for egg production
		(a) Raw squilla meal in layers (Experiment 3)
		(b) Raw and autoclaved squilla meal and raw squilla supplemented with enzyme in layers (Experiment 4)

3.1.1.1 Proximate and mineral composition

Proximate principles, calcium and phosphorus contents were determined by AOAC (1990) methods and contents for magnesium, manganese, copper, zinc and iron were analysed by atomic absorption spectroscopy (Anon, 1983). The chitin content was determined by van soest acid detergent fibre method (Goering and Van Soest, 1970) as recommended by Stelmock *et al.* (1985).

3.1.1.2 Aflatoxin estimation

Samples were extracted for aflatoxins with aqueous acetone (Water 30: acetone 70, v/v) and concentrated in chloroform (Pons *et al.*, 1966). Quantitative determination of aflatoxin was carried out with velasco fluorotoxin meter and values were obtained to the nearest ppb.

3.1.1.3 Amino acid composition

Amino acid analysis was carried on LKB automatic amino acid analyser (model 4400, LKB instruments,Switzerland) using samples hydrolysed with 6 N HCl for 24 h at 110^{o}C. The amino acid content of these samples was expressed as g per 16 g of nitrogen.

Methionine was determined by the method of Horn *et al.* (1946). Fat free samples of the squilla meal and fish meal were hydrolysed with 6 N HCl at 110^{o}C for 18 h. An aliquot of the hydrolysate in alkaline medium developed blue

colour with sodium nitro-prusside which was read at 540 mμ in a digital spectrophotometer. A regression equation was developed using pure DL-methionine treated similar to the test samples. The methionine content in the samples was calculated using the following equation:

$$Y = -0.057 + 5.393X,$$

where,

Y = concentration of methionine (mg) per ml of hydrolysate;

X = optical density.

Tryptophan was determined by the method of Spies and Chamber (1948). Fat-free samples of squilla meal and fish meal were hydrolysed with 6 N NaOH at 110°C for 16 h. The alkaline hydrolysate in presence of para-dimethyl-amino benzaldehyde (PDAB) and sodium nitrite developed a blue colour which was read at 560 mμ in a spectro-photometer. A regression equation was constructed with pure tryptophan treated similar to the samples. The tryptophan content of the samples was calculated using the following regression equation:

$$Y = 0.0071 + 0.1804X$$

where,

Y = mg of tryptophan per ml of hydrolysate,

X = optical density.

The available lysine of the squilla meal and fish meal was determined by the method of Rao *et al.* (1963). An aliquot of the hydrolysate was eluted first with 3 N HCl followed by a mixture of methylethyl ketone : 3 N HCl (1:3 v/v) through a chromatographic column prepared with amberlite IR-120 Na-form (size B). Yellow coloured epsilon-DNP-lysine was separated from dinitrophenol, other yellow components and brown humin pigments of acid hydrolysate and the absorbance was read at 435 nm in a digital spectrophotometer. A regression equation was developed with pure yellow epsilon DNP-lysine HCl. H_2O eluted similar to the test samples. The available lysine content of the test meterials was calculated using the regression equation:

$$Y = 0.0027 + 1.2016\ X$$

where,

Y = concentration of lysine in mg per 2 ml of hydrolysate

X = optical density.

3.1.2 *In vivo* studies

Gross protein value, proitein digestibility and metabolizable energy of squilla meal and fish meal were estimated for comparative evaluation of their nutritive value through biological experiments.

3.1.2.2 Determination of protein digestibility or absorbability

Protein digestibility was measured by the method of Lodhi *et al.* (1970). The protein retained in the terminal fifth segment of the small intestine was considered as unabsorbed protein.

Diet formulated using whole fish meal as sole protein source served as a reference diet, while test diet was formulated employing squilla meal at the expense of fish meal (Table 5). The two diets made isocaloric and isonitrogenous by adjusting suitable quantities of starch and saw dust. Chromic oxide was included in the diets at 8 g per kg. The chicks were housed in battery brooders and fed upto 7 d age on reference diet containing fish meal as protein source. Feed and water were provided *ad lib.* On 8th day the chicks were randomised on uniform body weight basis into six groups. Each of the two diets was fed to triplicate groups of six commercial broiler male chicks from 8 d to 35 d age.

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The weekly body weights were recorded during experimental period. At the end of experiment (35 d), 5 birds from each replicate group were sacrificed with choloroform and their digestive tracts were removed immediately. The contents of the terminal fifth segment of the small intestine (from the proximal end of the duodenum to the junction of the caecum and colon) were

Table 5: Composition of diets for determination of protein absorbability from fish meal and squilla meal

Ingredient, g/kg	Reference diet	Fish meal diet (control)	Squilla meal diet
Fish meal	238.0	238.0	-
Squilla meal	-	-	383.0
Starch	613.0	613.0	564.0
Chromic oxide	-	8.0	8.0
Saw dust	128.0	120.0	21.5
L-lysine HCl	-	-	2.0
DL-Methionine	-	-	0.5
Mineral mixture[1]	20.0	20.0	20.0
Vitamin and additive premix[2]	1.0	1.0	1.0
Nitrogen (analysed)	21.0	20.9	21.0
ME (calculated), MJ/kg	12.02	12.02	12.03

1. Mineral mixture supplied per kg diet: Calcium, 11 g; phosphorus, 6 g; sodium chloride, 6 g; potassium, 0.5 g; manganese, 79 mg; magnesium, 0.20 g; iron, 56 mg; zinc, 50 mg; copper, 2 mg; iodine, 2 mg and selenium, 0.09 mg.

2. Vitamin and additive premix supplied per kg diet: Vitamin A, 10000 IU; vitamin D_3, 1500 ICU; vitamin E 15 IU; thiamine hydrochloride, 10 mg; riboflavin, 10 mg; nicotinic acid, 80 mg; folic acid, 3 mg; pyridoxine, 20 mg; biotin, 0.3 mg; vitamin B_{12}, 6 mcg; choline, 3 mg; aureomycin, 10 mg and amprolium hydrochloride 25%, 125 mg.

immediately collected using distilled water and gentle pressure, avoiding as far as possible, shedding of epithelial tissue into the contents of the gut. The intestinal contents were mixed with few ml of 1 N sulfuric acid and dried at 60°C.

The dried samples of intestinal contents as well as feed were analysed for chromic oxide by Hill and Anderson (1958) and nitrogen by the AOAC (1990) methods. The un-absorbed nitrogen was first determined by comparing ratio of nitrogen to chromic oxide in the intestinal contents. The per cent nitrogen absorbed was calculated by deducting the per cent unabsorbed nitrogen from 100.

3.1.2.3 Metabolizable energy determination

Fish meal and squilla meal were included at the expense of a portion of a low protein basal diet at 200 and 400 g per kg levels. A separate premix of minerals and vitamins (Table 6) was added at 30 parts over and above 1000 parts for the diets, chromic oxide was included in diet at 3 g per kg.

Fifteen groups of 8 White Leghorn male chicks were reared in battery brooders from day old to 14 days of age offering standard farm diet and water *ad lib.* During the experimental period (15 d to 28 d) the five test diets (basal, 200 and 400 g per kg squilla meal and 200 and 400 g per kg fish meal) were offered to triplicate groups.

Table 6: Composition of basal diet and additive mixture used for the determination of metabolizable energy content of fish meal and squilla meal

Basal diet		Additive mixture	
Ingredient (fresh basis)	(g/kg)	Ingredient	(g/kg)
Yellow maize	750	Mineral mixture[1]	25.5
Rice polish	150	Chromic oxide	3.0
Fish meal	100	Vitamin & coccidiostat premix[2]	1.5
Total	1000		30.0

Nutrient composition (DMB)

Crude protein (analysed) (g/kg)	133.5
ME (calculated) (MJ/kg)	12.02

1. Mineral mixture supplied per kg diet: Calcium, 8.1 g; phosphorus, 1.5 g; copper, 2.6 mg; cobalt, 1.5 mg; manganese, 68.9 mg; iodine, 2.6 mg; zinc, 66.3, mg and iron, 25.5 mg.

2. Vitamin and coccidiostat premix supplied per kg diet; Vitamin A, 15000 IU; vitamin D3, 2000 IU; vitamin E, 16 IU; thiamine, 1.6 mg; riboflavin, 8 mg; pyridoxine, 3.2 mg; niacin, 24 mg; calcium pantothenate, 16 mg; vitamin B_{12} 20 mcg and amprolium hydrochloride 25%, 125 mg.

Excreta collections were done on last three days (26, 27 and 28 d age) of experimental period. Excreta collections were pooled replicate wise. Feed and excreta samples were processed as per the method of Sibbald and Slinger (1963a). Moisture and nitrogen were determined by AOAC (1990) method. Gross energy of the samples was determined by adiabatic bomb calorimeter and chromic oxide content in the feed and excreta was determined by the method of Hill and Anderson (1958). The nitrogen corrected metabolizable energy (MEn) of the diets was calculated as follows:

$$\text{MEn/g feed} = \text{GE/g feed} - \left(\frac{Cr_2O_3\text{/g feed}}{Cr_2O_3\text{/g excreta}} \times \text{GE/g excreta}\right)$$

$$\text{(N/g feed)} - \left(\frac{Cr_2O_3\text{/g feed}}{Cr_2O_3\text{/g excreta}} \times \text{N/g excreta}\right) \times 8.73$$

PART - II

3.2 UTILIZATION OF SQUILLA MEAL IN CHICKEN

The efficacy of squilla meal in replacing fish meal from broiler and layer diets was studied in four different experiments. In first two experiments (Experiment 1 and 2) utilization of squilla meal for growth in broilers was evaluated, while in other two experiments (Experiment 3 and 4) its utilization in white leghorn layers was assessed.

The experimental diets for all the four experiments were formulated replacing squilla meal with fish mixture in the control diet. The fish mixture contained, 620 g fish meal, 130 g starch, 24.8 g dicalcium phosphate, 64.7 g shellgrit, 15.8 g of salt and saw dust 144.7 g per kg. The fish mixture was thus made isonitrogenous and isocaloric to that of one kg of squilla meal which enabled to formulate test diets replacing part by part of fish mixture with squilla meal in control diet. All the experimental diets were analysed for crude protein as per AOAC (1990) methods.

3.2.1 Utilization of squilla meal for growth (Experiment 1 and 2)

In experiment 1, squilla meal was used at graded levels replacing fish mixture in order to evaluate the optimum level of inclusion of squilla meal in broiler diet, without affecting the growth performance. In a subsequent study (Experiment 2) the possibility of complete replacement of fish mixture with squilla meal by autoclaving or enzyme supplementing the test material was explored.

The biological trials on broilers were conducted in battery brooders. Standard managemental practices were followed. Feed and water were provided *ad lib*. During the experimental period, individual body weights and group feed

intake were recorded at weekly intervals. In all the experiments mortality was recorded through out the experiment period.

3.2.1.1 Experiment 1: Utilization of raw squilla meal in broilers (1 - 42 d age)

In this experiment, the influence of feeding raw squilla meal on the performance of broilers was studied. Day-old commercial broiler female chicks were weighed individually and distributed in to 15 groups of 8 chicks each. Three groups with uniform body weight were allotted at random to each of the dietary treatments. The control diet was formulated to contain 166.8 g per kg fish mixture. Four experimental diets were formulated (Table 7) incorporating squilla meal at 41.7, 83.4, 125.1 and 166.8 g per kg, at the expense of fish mixture in control diet, such that all the diets were isocaloric and isonitrogenous (Table 8).

The experiment lasted for 42 days and at the end of the experiment, visceral organ weights and lengths of different segments of intestine were measured. Histopathological changes, if any, in intestinal segments were also studied to examine the influence of chitin in test material on the digestive tract.

Table 7: Composition of diets with varying levels of squilla meal to study the performance of broiler chicks (1-42 d, experiment 1)

Ingredient, g/kg	Inclusion levels of squilla meal (g/kg)				
	0 (Control)	41.7	83.4	125.1	166.8
Maize	622.0	622.0	622.0	622.0	622.0
Soyabean cake	200.0	200.0	200.0	200.0	200.0
Fish mixture*	166.8	125.1	83.4	41.7	-
Squilla meal	-	41.7	83.4	125.1	166.8
Dicalcium phosphate	8.0	8.0	8.0	8.0	8.0
Mineral mixture[1]	1.0	1.0	1.0	1.0	1.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0
DL methionine	1.2	1.2	1.2	1.2	1.2

* Fish mixture contained: Fish meal, 620; starch, 130; dicalcium phosphate, 24.8; shell grit, 54.7; salt, 15.8 and saw dust, 144.7 g/kg.

1. Mineral mixture supplied per kg diet : Manganese, 79 mg; iron, 38 mg; zinc, 57 mg; copper, 4 mg; iodine, 0.99 mg and selenium, 0.08 mg.

2. Vitamin mixture supplied per kg diet : Vitamin A, 10000 IU; riboflavin, 6 mg; vitamin D_3, 1250 ICU; vitamin B_{12}, 20 mcg; vitamin E, 20 mg; thiamine, 6 mg; vitamin K, 0.5 mg; biotin, 0.15 mg; calcium pantothenate, 15 mg; pyrodoxine, 3 mg; niacin, 40 mg, folic acid, 0.5 mg and amprolium hydrochloride 25%, 125 mg.

\- Not included

Table 8: Nutrient composition of diets (g per kg) with varying levels of squilla meal to study the performance of broilers chicks (1-42d, experiment 1)

Nutrient, g/kg	Inclusion levels of squilla meal (g/kg)				
	0 (Control)	41.7	83.4	125.1	166.8
Crude protein (analysed)	210.00	211.00	210.00	212.00	211.00
Calculated values					
ME, (MJ/kg)	11.76	11.76	11.77	11.78	11.79
Ether extract	28.60	28.60	28.50	28.40	28.30
Total ash	59.80	67.60	75.60	83.50	91.20
Calcium	13.60	13.50	13.50	13.50	13.50
Available phosphorus	5.10	5.10	5.00	5.00	5.00
Lysine	10.20	9.90	9.50	9.10	8.70
Methionine	4.45	4.40	4.30	4.20	4.10

3.2.1.2 Experiment 2: Utilization of raw and autocalved squilla meal and raw squilla meal supplemented with enzyme for broilers (8 - 42 d age)

In this experiment, the influence of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on the performance of broilers was studied. The raw and autoclaved squilla meal were used at three levels (83.4, 125.1 and 166.8 g/kg) at the expense of fish mixture, thus forming six treatments. Autoclaving at 1.09 kg/cm^2 pressure for 5 mt, the autoclaved material was sun dried and used. Enzyme premix XP33 (a proprietary product of Finnfeeds International, UK, containing 10,000 units/g of bacterial proteases) was supplemented at three graded levels (0.5, 1.0 and 2.0 g/kg diet) for the above three raw squilla meal levels, thus forming nine treatments. The 15 treatment groups were compared with control (fish mixture based diet) making an overall total of 16 treatments (Table 9).

The day-old female broiler (450) chicks were weighed individually and reared in battery brooders upto 8 days of age on standard starter diet. On 8th day 336 chicks were selected by McKittrick technique (McKittrick, 1947), and were allotted to 48 groups of 7 chicks each. Three groups were assigned to each of the 16 dietary treatments. Experimental diets, which were iso-nitrogenous and iso-caloric (Table 10), were offered during the experimental period (8 - 42 days).

Table 9: Composition of diets with varying levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme to study the performance of broiler chicks (8-42d, experiment 2)

Ingredient, g/kg	Inclusion levels of squilla (g/kg)						
	D_1 0(control)	D_2 83.4	D_3 125.1	D_4 166.8	D_5 83.4	D_6 125.1	D_7 166.8
Maize	622.0	622.0	622.0	622.0	622.0	622.0	622.0
Soyabean cake	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Fish mixture	166.8	83.4	41.7	-	83.4	41.7	-
Squilla meal	-	83.4	125.1	166.8	-	-	-
Squilla meal (autoclaved)	-	-	-	-	83.4	125.1	166.8
DCP	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Mineral mixture[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL methionine	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Enzyme premix added to raw squilla meal diets:							
a) 0.5 g/kg diet	-	+ (D_8)	+ (D_9)	+ (D_{10})	-	-	-
b) 1.0 g/kg diet	-	+ (D_{11})	+ (D_{12})	+ (D_{13})	-	-	-
c) 2.0 g/kg diet	-	+ (D_{14})	+ (D_{15})	+ (D_{16})	-	-	-

1. Mineral mixture supplied per kg diet : Same as given in table 7
2. Vitamin mixture supplied per kg diet : Same as given in table 7

\+ Enzyme premix included in the diet

\- Not included

Table 10: Nutrient composition of diets (g/kg) with varying levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme to study the performance of broiler chicks (8-42 d, experiment 2).

Nutrient, g/kg	Inclusion levels of squilla meal (g/kg)															
	D_1 0(control)	D_2 83.4	D_3 125.1	D_4 166.8	D_5 83.4	D_6 125.1	D_7 166.8	D_8 83.4	D_9 125.1	D_{10} 166.8	D_{11} 83.4	D_{12} 125.1	D_{13} 166.8	D_{14} 83.4	D_{15} 125.1	D_{16} 166.8
Crude protein (analysed)	211.00	210.00	211.00	211.00	209.00	210.00	209.00	210.00	211.00	209.00	211.00	209.00	210.00	211.00	210.00	209.00
Calculated values																
ME, (MJ/kg)	11.76	11.77	11.78	11.79	11.77	11.78	11.79	11.77	11.78	11.79	11.77	11.78	11.79	11.77	11.78	11.79
Ether extract	28.60	28.50	28.40	28.30	28.50	28.40	28.30	28.50	28.40	28.30	28.50	28.40	28.30	28.50	28.40	28.30
Calcium	13.60	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Available phosphorus	5.10	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lysine	10.20	9.50	9.10	8.70	9.50	9.10	8.70	9.50	9.10	8.70	9.50	9.10	8.70	9.50	9.10	8.70
Methionine	4.45	4.30	4.20	4.10	4.30	4.20	4.10	4.30	4.20	4.10	4.30	4.20	4.10	4.30	4.20	4.10

3.2.2 Utilization of squilla meal for egg production (Experiment 3 and 4)

In experiment 3, squilla meal was used at graded levels replacing fish mixture in order to evaluate the optimum level of incorporation of squilla meal in layer diet without affecting the egg production. In subsequent study (Experiment 4) the possibility of complete replacement of fish mixture with squilla meal by autoclaving or enzyme supplementing the test material was examined.

The biological trails on White Leghorn layers were conducted in cages. Standard managemental practices were followed. Feed and water were provided *ad lib.*

3.2.2.1 Experiment 3: Utilization of raw squilla meal in layers

In this experiment, the influence of feeding squilla meal in diets on laying performance was examined. The control diet was formulated to contain 133.4 g/kg fish mixture. Three experimental diets were formulated (Table 11) incorporating squilla meal at 44.5, 88.9 and 133.4 g/kg at the expense of fish mixture in control diet, such that all the diets were isonitrogenous and isocaloric. The diets were fed to triplicate groups of six White Leghorn layers per group, from 30-45 wk age. The layers of each replicate were placed in three adjacent cage cells with two layers per cage

Table 11: Composition of diets with varying levels of squilla meal to study the performance of WL layers (30-45 wk, experiment 3)

Ingredient, g/kg	Inclusion levels of squilla meal (g/kg) (Control) 0	44.5	88.9	133.4
Maize	570.0	570.0	570.0	570.0
Sunflower cake	120.0	120.0	120.0	120.0
Groundnut cake	100.0	100.0	100.0	100.0
Fish mixture*	133.4	88.9	44.5	-
Squilla	-	44.5	88.9	133.4
Shell grit	66.0	66.0	66.0	66.0
Dicalcium phosphate	8.6	8.6	8.6	8.6
Mineral mixture[1]	1.0	1.0	1.0	1.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0
Nutrient composition:				
Crude protein (analysed)	169.2	170.1	168.6	169.5
ME, (MJ/kg, calculated)	10.97	10.97	10.98	10.99
Crude fibre (calculated)	62.80	65.20	67.10	69.90
Total ash " " "	63.90	72.20	80.60	89.00
Calcium " " "	36.4	36.3	36.2	36.1
Available phosphorus "	4.8	4.7	4.6	4.5

* Fish mixture contained: Fish meal, 620; starch, 130; dicalcium phosphate, 24.8; shell grit, 64.7; salt, 15.8 and saw dust, 144. g/kg.

1. Mineral mixture supplied per kg diet : Manganese, 66 mg; zinc, 52 mg; iron, 40 mg; copper, 3.0 mg; iodine, 0.74 mg and selenium, 0.08 mg.

2. Vitamin mixture supplied per kg diet : Vitamin A, 10000 IU; riboflavin, 5 mg; vitamin D_3, 1250 ICU; vitamin B_{12}, 15 mcg; vitamin E, 16 mg; thiamine, 5 mg; vitamin K, 0.5 mg; pyridoxine, 3 mg; calcium pantothenate, 15 mg; biotin, 0.15 mg

cell providing common feeders. The experiment lasted for four periods of 28 days each.

3.2.2.2 Experiment 4: Utilization of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme in layers

In this experiment, the influence of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on the performance of layers was studied. The raw and autoclaved squilla meal were used at two levels (88.9 and 133.4 g/kg) at the expense of fish mixture, thus forming four treatments. Enzyme premix was supplemented at three graded levels (0.5, 1.0 and 2.0 g/kg) for the above two raw squilla levels, thus forming six treatments. The ten treatment groups were compared with control, making a total of 11 treatment groups (Table 12 and 13).

Each diet was fed to triplicate groups of four White Leghorn layers per group, from 25 wk to 40 wk age i.e., 4 x 28 d periods. The layers of each replicate were placed in two adjacent cells with two layers per cage cell providing a common feeder. All the birds prior to the experimentation were reared on deep litter under standard farm managemental conditions and were fed on a common standard chick and grower diets.

During the trials (Experiment 3 and 4), daily egg production and period-wise feed intake for each replicate

Table 12: Composition of diets with varying levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme to study the performance of WL layers (25-40 wk, experiment 4)

Ingredient, g/kg	Inclusion levels of squilla meal (g/kg)				
	D_1 0(control)	D_2 88.9	D_3 133.4	D_4 88.9	D_5 133.4
Maize	570.0	570.0	570.0	570.0	570.0
Sunflower cake	120.0	120.0	120.0	120.0	120.0
Groundnut cake	100.0	100.0	100.0	100.0	100.0
Fish mixture	133.4	44.5	-	44.5	-
Squilla meal	-	88.9	133.4	-	-
Squilla meal (autoclaved)	-	-	-	88.9	133.4
Shell grit	66.0	66.0	66.0	66.0	66.0
DCP	8.6	8.6	8.6	8.6	8.6
Mineral mixture[1]	1.0	1.0	1.0	1.0	1.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0
Enzyme premix added to raw squilla diets :					
a) 0.5 g/kg diet	-	+ (D6)	+ (D7)	-	-
b) 1.0 g/kg diet	-	+ (D8)	+ (D9)	-	-
c) 2.0 g/kg diet	-	+ (D10)	+ (D11)	-	-

1 Mineral mixture supplied per kg diet : As given in table 11
2 Vitamin mixture supplied per kg diet : As given in table 11
+ Enzyme premix included in the diet
- N included

Table 13: Nutrient composition of diets (g/kg) with varying levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme to study the performance of WL layers (25-40 wk, experiment 4)

Nutrient, g/kg	Inclusion levels of squilla meal (g/kg)										
	D_1 0(control)	D_2 88.9	D_3 133.4	D_4 88.4	D_5 133.4	D_6 88.9	D_7 133.4	D_8 88.9	D_9 133.4	D_{10} 88.9	D_{11} 133.4
Crude protein (analysed)	169.4	169.6	170.1	169.8	169.6	168.8	169.2	169.6	169.4	169.8	169.7
Calculated values											
ME, (MJ/kg)	10.97	10.98	10.99	10.98	10.99	10.98	10.99	10.98	10.99	10.98	10.98
Crude fibre	62.80	67.10	69.90	67.10	69.90	67.10	69.90	69.10	69.90	67.10	69.90
Total ash	63.90	80.60	89.00	80.60	89.00	80.60	89.60	80.60	89.60	80.60	89.60
Calcium	36.40	36.20	36.10	36.20	36.10	36.20	36.10	36.20	36.10	36.20	36.10
Phosphorus (available)	4.80	4.60	4.50	4.60	4.50	4.60	4.50	4.60	4.50	4.60	4.50

group were recorded. On the last three days (26, 27 and 28 d) of each laying period nine eggs per day per treatment (Experiment 3) six eggs per day per treatment (Experiment 4) were collected for evaluating external and internal egg quality. Parameters like egg weight, albumen index, yolk index, Haugh unit score and shell thickness were determined. The mean replicate value of each day were considered for statistical analysis.

3.2.3 Statistical analysis

The data were subjected to statistical analysis (Nageswara Rao, 1983). One way classification of analysis of the variance (completely randomised design) was carried out on the data on body weight, feed intake, feed to gain ratio, feed cost to produce per kg live weight, visceral organ weights and length of individual segments of gastro-intestinal tract (Experiment 1), livability (Experiment 2). Regression analysis (Snedecor and Cochran, 1967) was done on the data on body weight, feed intake, feed to gain ratio (Experiment 1 and 2) visceral organ weights and length of individual segement of gastrointestinal tract (Experiment 1).

Factorial randomized block design analysis was done on the data on body weight, feed intake, feed to gain ratio (Experiment 2), hen day egg production, feed intake, feed efficiency and egg quality parameters (Experiment 3 and 4).

CHAPTER IV

RESULTS

The results of the investigations on nutrient composition of squilla meal in comparison to that of fish meal are presented in part I. The utilization of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme in poultry is presented in Part II.

PART - I

4.1 NUTRITIVE VALUE OF SQUILLA MEAL

The nutrient composition of squilla meal was evaluated by *in vitro* methods in terms of proximate principles, trace minerals and amino acid profile; and by *in vivo* methods in terms of gross protein value, protein digestibility and metabolizable energy content.

4.1.1 *In vitro* studies

4.1.1.1 Proximate principles and trace mineral composition

The data on nutrient composition of squilla meal and fish meal are reported in Table 14. Squilla meal was lower in crude protein and ether extract contents than fish meal but, higher in crude fibre, nitrogen free extract and total ash. Both were comparable in terms of calcium and phosphorus content. The content of magnesium, manganese, zinc and iron were more in squilla meal than in fish meal.

Table 14: Proximate and mineral compositior of squilla meal and fish meal (g/kg, DMB)

Constituent	Squilla meal	Fish meal
Crude protein	339.7	546.0
Ether extract	18.6	32.8
Crude fibre	139.2*	32.0
Nitrogen-free-extract	119.4	74.7
Total ash	383.1	314.5
Mineral composition:		
Calcium	72.8	66.4
Phosphorus	17.2	15.8
Magnesium	5.7	2.4
Zinc, (ppm)	450	350
Manganese (ppm)	48	24
Iron (ppm)	1000	750
Copper (ppm)	100	96

* Chitin

4.1.1.2 Aflatoxins

Both the squilla meal and fish meal used in these studies were free of aflatoxins.

4.1.1.3 Amino acid profile

The squilla meal was lower in the content of amino acids than fish meal (Table 15), except for tyrosine (2.35 Vs 2.30 g/16 g N). The total and available lysine content of squilla protein were 3.45 and 2.38 g per 16 g N, respectively whereas, methionine, arginine and tryptophan were 1.02, 2.37 and 0.65 g per 16 g N, respectively.

4.1.2 *In vivo* studies

Protein quality of squilla meal assessed as GPV and protein digestibility, along with its metabolizability and ME values determined in three separate experiments are presented below.

4.1.2.1 Gross protein value

The weight gain per g of squilla and fish protein during the test period were 8.39 and 8.72 g respectively as against 11.02 g recorded with the reference protein (casein). The gross protein values of squilla meal and fish meal accounted for 68.00 and 72.02 per cent respectively (Table 16).

Table 15: Amino acid composition (g/16 g N) of squilla meal and fish meal

Amino acid	Squilla meal	Fish meal
Aspartic acid	6.31	7.21
Threonine	2.79	4.38
Serine	2.24	3.36
Glycine	4.93	5.37
Glutamic acid	10.99	13.01
Proline	3.48	4.32
Alanine	3.95	4.94
Valine	3.55	4.31
Methionine	1.02	1.86
Isoleucine	3.32	4.04
Leucine	5.05	6.13
Tyrosine	2.35	2.30
Phenylalanine	2.64	3.50
Tryptophan	0.65	0.82
Histidine	1.20	1.48
Lysine	3.45	6.74
Lysine (available)	2.38	4.79
Arginine	2.37	5.57

Table 16: Gross protein value of squilla meal and fish meal for broiler chicks

	Depletion diet	Casein diet	Squilla meal diet	Fish meal diet
Mean weight gain, g	57.82	103.36	74.41	76.38
Total protein consumed/bird, g	20.65	34.60	30.76	31.97
Supplementary protein consumed/bird, g	-	9.38	8.87	8.76
(a) Gain/g of supplementary protein consumed	-	11.02	8.39	8.72
(b) Gain/g of protein consumed in depletion diet	2.80	-	-	-
GPV (%) ±SE		100.00	68.00 ±0.98	72.02 ±1.12

± Standard error

$$GPV = \frac{\text{a-b of test protein}}{\text{a-b of casein protein}} \times 100$$

4.1.2.2 Protein digestibility or absorbability

The N digestibility data determined with terminal fifth segment of the small intestine by the method of Lodhi *et al.* (1970) for squilla meal and fish meal is presented in Table 17. Squilla meal had lower 'N' digestibility than the fish meal used in the studies.

4.1.2.3 Metabolizable energy

The metabolizable energy content of squilla meal and fish meal determined by the practical diet method of sibbald and Slinger (1963), including the test materials at 200 and 400 g/kg diet is presented in Table 18. The AMEn content at both the levels of inclusion differed only by 1.5 to 2.0 per cent. Squilla meal had lower AMEn than fish meal (7.13 Vs 8.08 MJ/kg).

Table 18: Metabolizable energy content (MJ/kg) of Squilla meal and fish meal by biological method (DMB)

	Level of basal diet replacement (g/kg)	Nitrogen corrected* ME (MJ/kg)	Mean AMEn (MJ/kg)
Squilla meal	200	7.08	7.13
	400	7.18	
Fish meal	200	8.00	8.08
	400	8.16	

* Mean of 3 replicate groups

Table 17: Nitrogen absorbability of squilla meal and fish meal

Treatment	N content (g/kg)		Cr_2O_3 content (g/kg)		Ratio of nitrogen to Cr_2O_3 in		**% nitrogen absorbability $= 100 - (\frac{b}{a} \times 100)$
	Feed	Intestinal contents**	Feed	Intestinal contents**	Feed (a)	Intestinal contents** (b)	
Squilla meal	20.8	16.3	7.93	18.27	2.623	0.891	66.03 ± 0.9
Fish meal	20.7	15.1	7.80	21.84	2.654	0.690	74.00 ± 0.6

* Means of three replicate groups
** Terminal fifth segment
± Standard Error

PART - II

4.2 UTILIZATION OF SQUILLA MEAL IN CHICKEN

The data on the utilization of squilla meal for growth and egg production in chicken are presented in this section.

4.2.1 Utilization of squilla meal for growth (Experiment 1 and 2)

Results obtained on inclusion of squilla meal replacing fish mixture at four levels (41.7, 83.4, 125.1 and 166.8 g/kg) in diet (Experiment 1) and the inclusion of raw and autoclaved squilla meal at 83.4, 125.1 and 166.8 g/kg and the influence of supplementation of enzyme premix (0.5, 1.0 and 2.0 g/kg diet) to above three raw squilla meal diets (Experiment 2) are described.

4.2.1.1 Experiment 1: Utilization of raw squilla meal in broilers (1-42 d age)

4.2.1.1.1 Body weight: The performance of female broilers on different levels of squilla meal in diet is presented in Table 19 and depicted in Fig.3. The body weight gains on fish meal and different levels of squilla meal in diet were comparable, though the higher level of squilla meal yielded appreciably lower body weight gains than other diets.

4.2.1.1.2 Feed intake and feed efficiency: Feed intake as well as feed efficiency were not influenced significantly (P>0.05) by inclusion of squilla meal in broiler diets (Table 19 and Fig.3), though numerically higher feed intake and poorer feed efficiency were observed with increasing levels of dietary squilla meal.

Regression equations of the level of squilla meal on the performance of broilers (Table 19) revealed a negative influence on weight gain, feed efficiency and increment on feed intake on the increasing levels of squilla meal in diet.

4.2.1.1.3 Economics: The feed cost/kg live weight gain with all levels of inclusion of squilla meal was comparable to that on control diet (Table 19). However, the feed cost was slightly higher with diets containing squilla meal at 41.7 and 83.4 g/kg.

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4.2.1.1.4 Weights of visceral organs: The weights of visceral organs viz., liver, spleen, kidney and pancreas expressed as per cent of body weight are presented in Table 20. Squilla meal and fish meal were similar in influencing the weights of these organs. Regression analysis revealed insignificant linear effect on all organ weights.

4.2.1.1.5 Length of intestinal segments: The length of intestinal segments viz., Duodenum, (Jejunum + ileum) and caecum were similar on diets containing different levels of

Table 19: Effect of varying levels of dietary squilla meal on performance of broiler chicks (1-42d, experiment 1)

Squilla meal, g/kg	Body weight gain, g/bird	Feed intake g/bird	Feed to gain ratio	Feed cost to produce per kg live wt. (Rs.)
0	1347±40	2905±39	2.16±0.05	12.51±0.42
41.7	1372±39	3143±80	2.29±0.09	12.89±0.68
83.4	1309±28	3195±28	2.44±0.02	13.35±0.12
125.1	1307±43	3079±45	2.35±0.05	12.51±0.37
166.8	1250±50	3058±98	2.42±0.02	12.57±0.18
Regression	1368 - 0.6211x	3028 + 0.5803x	2.216 + 0.0014x	--
Standard errors:				
Intecept	24.9660	118.8383	0.0762	--
Slope	0.1893	0.9012	0.00005	--
R^2	0.7820	0.1215	0.6585	--
P	0.0464	0.5655	0.0954	--

x Squilla meal (g/kg)
± Standard Error

Analysis of variance

Source of variance	Body weight gain		Feed intake		Feed to gain ratio		Feed cost to produce 1 kg live wt.	
	df.	MSS	df.	MSS	df.	MSS	df.	MSS
Due to treatment	4	42732	4	37492	4	0.0342	4	0.3996
Error	99	35563	10	54805	10	0.0218	10	0.4929

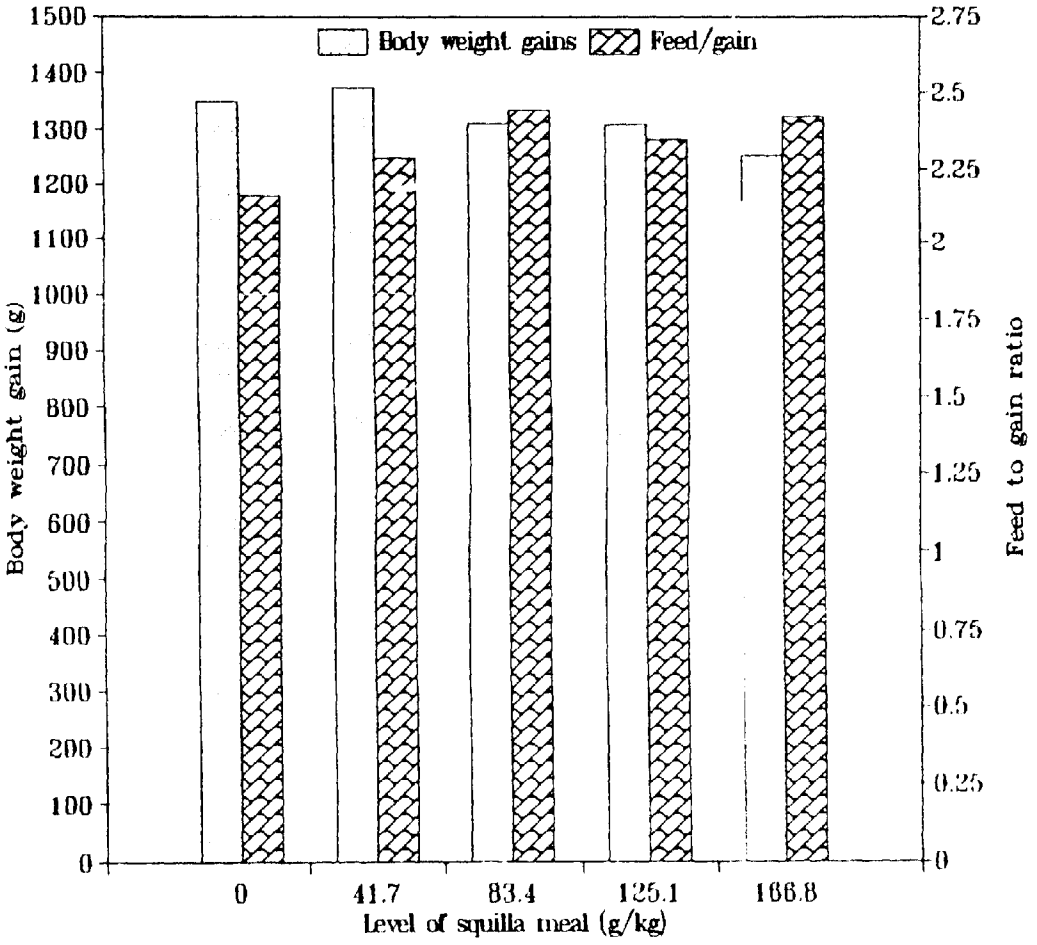

Fig.3. Performance of broilers (1–42 d age) on various levels of squilla meal (Expt.1)

Table 20: Effect of varying dietary levels of squilla meal or visceral organ weights of broilers (1-42 d, experiment 1)

Squilla meal, g/kg	Visceral organs (% of body weight)			
	Liver	Spleen	Kidney	Pancreas
0	2.65±0.012	0.245±0.017	0.544±0.054	0.320±0.028
41.7	3.04±0.221	0.242±0.012	0.445±0.034	0.272±0.011
83.4	2.90±0.143	0.213±0.011	0.447±0.039	0.299±0.030
125.1	2.99±0.096	0.220±0.013	0.507±0.031	0.298±0.024
166.8	2.75±0.162	0.247±0.021	0.527±0.019	0.306±0.021
Regression	2.836+0.00036x	0.2370-0.000043x	0.4884+0.000067x	0.2994-0.0000047x
Standard errors:				
Intecept	0.1868	0.0179	0.0526	0.0202
Slope	0.0014	0.000014	0.000398	0.000152
R^2	0.0210	0.0328	0.0094	0.0003
P	0.8159	0.7708	0.8769	0.9769

x = Squilla meal (g/kg)
± : Standard Error

Analysis of variance

Source of variance	df	Mean sum of squares			
		Liver	Spleen	Kidney	Pancreas
Due to treatments	4	0.1567	0.0015	0.0123	0.0018
Error	25	0.1382	0.0014	0.0082	0.0035

squilla meal (Table 21). However, the linearity in increase in the length of duodenum (from 18.1 to 20.8 cm) and caecum (from 10.8 to 12.5 cm) with the increasing levels of squilla meal inclusion was significant (P=0.0435 and P=0.0028, respectively). Histopathological studies revealed a slight increase in the number of goblet cells in the intestinal mucosa on squilla meal fed birds resulting in the thickening of the intestinal wall.

4.2.1.2 Experiment II: Utilization of raw and autoclaved squilla meal and raw squilla supplemented with enzyme in broilers (8-42 d age)

4.2.1.2.1 Body weight gain: The effect of treated squilla meal with autocalving and enzymes at different levels is presented in Table 22. The interaction of treatment and level of squilla meal was not significant (P>0.05). On diets containing squilla meal at 83.4 and 125.1 g/kg the body weight gains were similar to than that on reference diet. However, squilla meal at 166.8 g/kg depressed (P<0.05) body weight gains than on reference diet and diet containing squilla meal at 125.1 g/kg (Fig.4).

Irrespective of the level of inclusion of raw squilla meal, when replaced fish nitrogen appreciably lowered (P<0.01) weight gains (1360 g) than on control (1415 g).

Table 21: Effect of varying dietary levels of squilla meal on length of the individual segments of gastro-intestinal tract of broilers (1-42d, experiment 1)

Squilla meal, g/kg	Length (cm/kg body weight)		
	Duodenum	Jejunum+Ileum	Caecum
0 (Fish control)	18.1±0.7	86.2±5.3	10.8±0.8
41.7	17.2±0.8	86.2±1.6	11.5±0.5
83.4	18.8±0.9	83.6±3.1	11.9±1.5
125.1	19.7±1.0	83.4±5.9	12.1±0.6
166.8	20.8±1.8	91.1±5.1	12.5±0.9
Regression	17.348+0.0187x	84.7+0.0165x	10.964+0.0094x
Standard errors:			
Intercept	0.7320	3.3382	0.1372
Slope	0.0056	0.0253	0.0010
R^2	0.7906	0.1239	0.9645
P	0.0435	0.5605	0.0028

x Squilla meal (g/kg)

Analysis of variance

Source of variance	df	Mean sum of squares		
		Duodenum	Jejunum+Ileum	Caecum
Due to treatments	4	11.537	78.743	2.288
Error	25	7.580	112.314	5.038

Table 22. Effect of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on body weight gain in broiler chicks (8-42 d, experiment 2).

Squilla meal, g/kg	Body weight gain (g)						Mean + SE
	Squilla meal						
	Fish based control	Raw	Autoclaved	Raw + enzyme 0.5 g/kg	Raw + enzyme 1 g/kg	Raw + enzyme 2 g/kg	
0	1415	-	-	-	-	-	1415^{p} + 29
83.4	-	1380	1319	1497	1422	1388	1401^{pq} + 17
125.1	-	1377	1308	1439	1494	1450	1414^{p} + 22
166.8	-	1322	1291	1396	1414	1373	1359^{q} + 17
Mean+SE	1415^{ab}+29	1360^{bc}+13	1306^{c}+17	1444^{a}+20	1443^{a}+23	1404^{ab}+19	
Regression	-	1420-0.50017	1403-0.7482x	1446-0.09797x	1422+0.1527x	1417-0.1109x	
Standard Errors:							
Intercept	-	17.6432	20.2533	53.0220	45.4397	40.2588	
Slope	-	0.1430	0.1641	0.4298	0.3683	0.3263	
R^2	-	0.8594	0.9121	0.0253	0.0792	0.0546	
P	-	0.0729	0.0449	0.8408	0.7185	0.7661	

+ Standard error

Treatment means in a row or column with different superscripts are significantly ($P<0.05$) different.

x = Squilla meal (g/kg).

Analysis of variance

Source of variance	df	MSS
Between treatments	4	31372.00**
Due to levels	2	12097.00*
Treatments x levels	8	2537.00
Treatments Vs control	1	1524.00
Error	32	3301.00

** Significant ($P<0.01$) : * Significant ($P<0.05$)

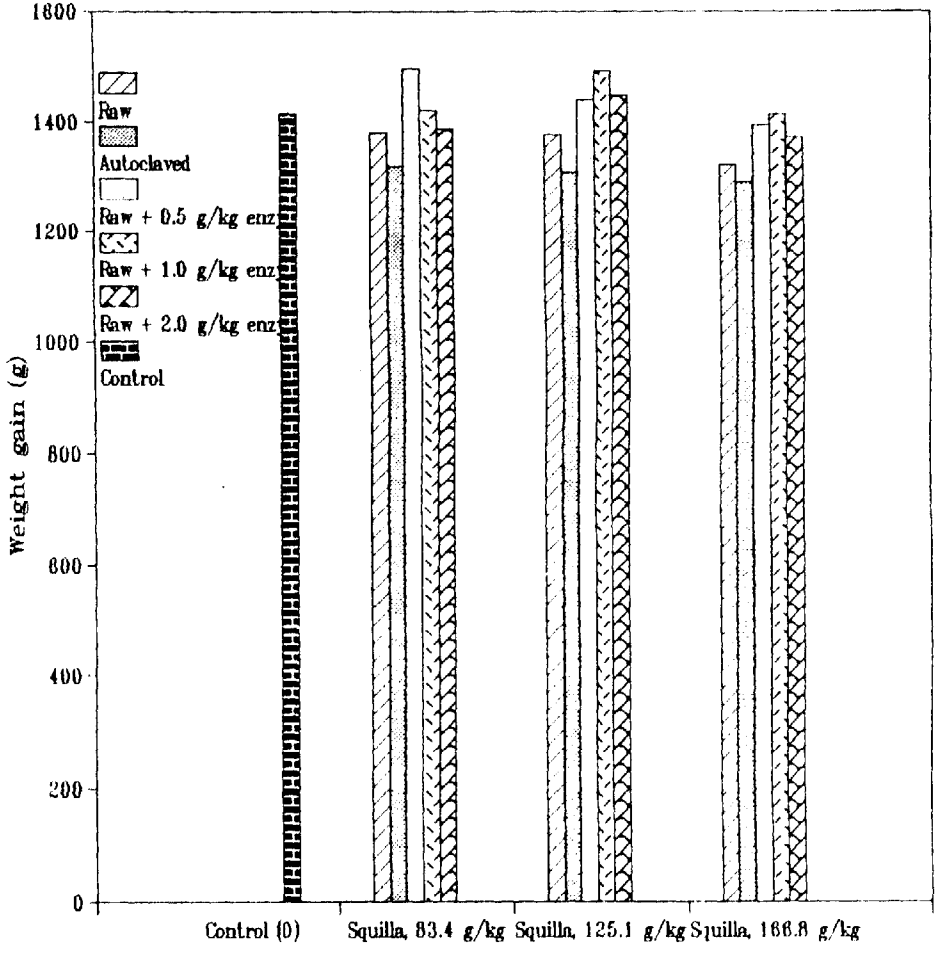

Fig.4. Body weight gain of broilers (42 d age) on variously treated squilla meal

Autoclaving of squilla meal, decreased body weight gains ($P<0.05$) compared with that on reference diet. The differences in body weight gains on raw and autocalved squilla meal are not singificantly different.

Enzyme supplementation at different levels did not influence body weight gain compared with the reference diet. However, enzyme supplementation at 0.5 and 1.0 g/kg improved weight gains than on raw squilla meal.

The regression equation of the level of squilla meal (Table 22) on growth indicated a negative influence of the increasing level on weight gain on raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme at 0.5 and 2.0 g/kg diet. However, the response with increasing level of squilla meal supplemented with enzyme at 1.0 g/kg was positive.

4.2.1.2.2 Feed intake and feed efficiency: There was an insignificant, numerically higher feed intake on all the treatment groups than control. Similar trend was observed for all inclusion levels of dietary squilla meal. The interaction of treatment and level of squilla meal had no effect on feed intake (Table 23). Regression equation was also confirmed the above trend.

The level of inclusion and treatment of squilla meal did not affect feed efficiency (Table 24 and Fig.5). The feed efficiency values on different levels of squilla

Table 23. Effect of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on feed intake in broiler chicks (8-42 d, experiment 2).

Squilla meal, g/kg	Feed intake (g)						Mean ± SE
		Squilla meal					
	Fish based control	Raw	Autoclaved	Raw + enzyme 0.5 g/kg	Raw + enzyme 1 g/kg	Raw + enzyme 2 g/kg	
0	2766	-	-	-	-	-	2766 ± 38
83.4	-	2985	2917	3014	2876	2778	2914 ± 43
125.1	-	2959	2873	2907	2739	2929	2881 ± 43
166.8	-	2883	2812	2816	2836	2946	2859 ± 29
Mean±SE	2766±38	2943±46	2867±34	2912±43	2817±81	2884±35	
Regression	-	2822+0.8078x	2810+0.3371x	2842+0.3597x	2785+0.2049x	2743+1.1901x	
Standard Errors:							
Intercept	-	97.4933	75.9574	129.8769	74.9606	55.0252	
Slope	-	0.7904	0.6156	1.0529	0.6077	0.4461	
R^2	-	0.3431	0.1304	0.0551	0.0538	0.7806	
P	-	0.4143	0.6389	0.7651	0.7681	0.1164	

± : Standard error
x = Squilla meal (g/kg).

Analysis of variance

Source of variance	df	MSS
Between treatments	4	20208.00
Due to levels	2	11680.00
Treatments x levels	8	18724.00
Treatments Vs control	1	39432.00
Error	32	27398.00

Table 24. Effect of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on feed to gain ratio in broiler chicks (8-42 d, experiment 2).

Squilla meal, g/kg	Feed to gain ratio						Mean ± SE
	Squilla meal						
	Fish based control	Raw	Autoclaved	Raw + enzyme 0.5 g/kg	Raw + enzyme 1 g/kg	Raw + enzyme 2 g/kg	
0	1.97	-	-	-	-	-	1.97 ± 0.01
83.4	-	2.16	2.21	2.01	2.02	2.00	2.08 ± 0.03
125.1	-	2.15	2.21	2.04	2.03	2.02	2.09 ± 0.03
166.8	-	2.15	2.18	2.05	2.00	2.15	2.11 ± 0.02
Mean±SE	1.97^{a}±0.01	2.16^{c}±0.02	2.20^{c}±0.02	2.04^{ab}±0.01	2.02^{ab}±0.03	2.06^{b}±0.03	
Regression	-	2.04+0.0011x	2.017+0.0013x	1.9 06+0.0005x	1.983+0.00002x	1.9463+0.00094x	
Standard Errors:							
Intercept	-	0.0581	0.0810	0.0059	0.0252	0.0515	
Slope	-	0.00047	0.00066	0.00005	0.00002	0.00004	
R^2	-	0.7325	0.6744	0.9823	0.3932	0.7917	
P	-	0.1441	0.1787	0.0088	0.3729	0.1516	

+ = Standard error
Treatment means in row with different superscripts are significantly (P<0.05) different.
x = Squilla meal (g/kg)

Analysis of variance

Source of variance	df	MSS
Between treatments	4	0.0578**
Due to levels	2	0.0025
Treatments x levels	8	0.0049
Treatments Vs control	1	0.0449
Error	32	0.0069

** Significant (P<0.01)

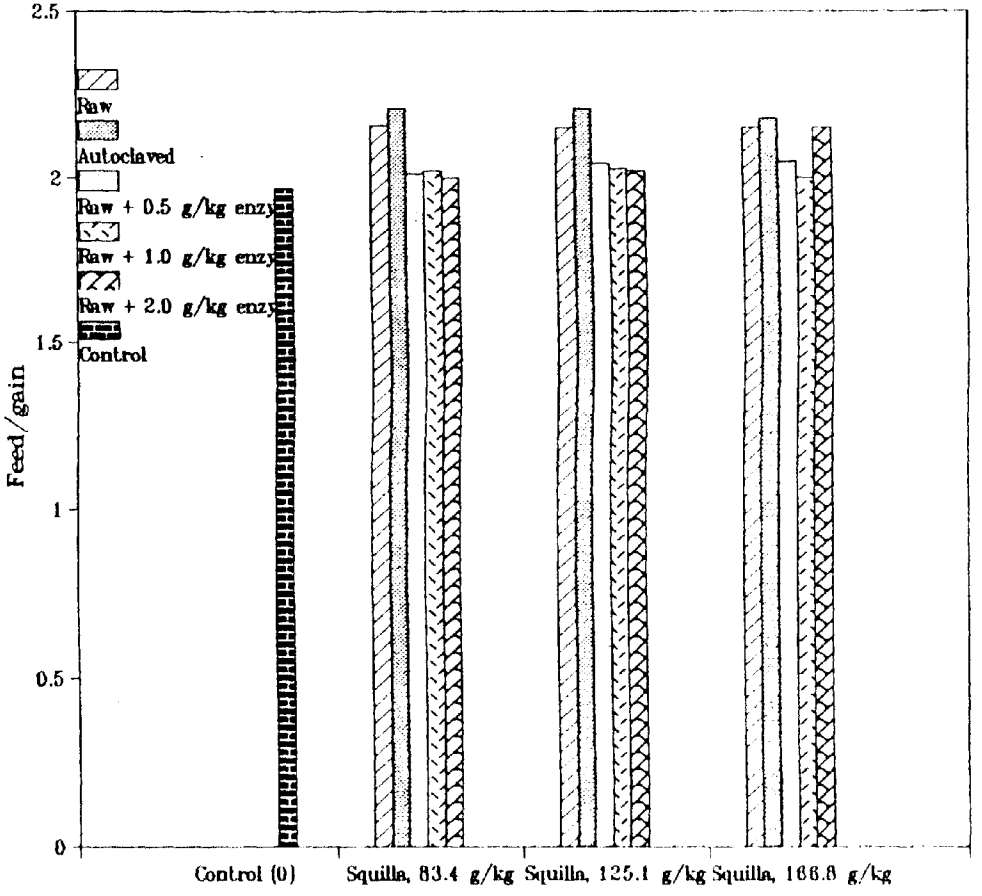

Fig.5. Feed to gain ratio of broilers (42 d age) on variously treated squilla meal

meal, though, were poorer than on reference diet, the values were comparable statistically.

Irrespective of dietary squilla meal levels, the feed efficiency values were influenced by dietary treatments being significantly ($P<0.05$) lower on autoclaved and raw squilla meal diets than on other diets. However, supplementation of enzyme premix at 0.5 to 2.0 g/kg diet improved the feed efficiency value. The feed efficiency value on enzyme supplementation at 0.5 and 1.0 g/kg (2.04 and 2.02) were comparable with that on control (1.97).

Regression analysis of the data revealed that feed efficiency values (Table 24) did decrease with increase in the level of squilla meal.

4.2.1.2.3 Livability: Livability on different diets was similar and ranged from 93.5 to 100 per cent (Table 25).

4.2.2 Utilization of squilla meal for egg production (Experiment 3 and 4)

Results obtained on the inclusion of squilla meal at 44.5, 88.9 and 133.4 g/kg replacing fish protein in layer diet are described in experiment 3. The inclusion of raw and autoclaved squilla meal at 88.9 and 133.4 g/kg and the influence of supplementation of enzyme premix (0.5, 1.0 and 2.0 g/kg diet) to the 88.9 and 133.4 g/kg raw squilla meal diets are described in experiment 4.

Table 25: Effect of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on livability of broiler chicks (8-42 d, experiment 2).

Diet	Inclusion levels of squilla meal (g/kg)			
	0	83.4	125.1	166.8
Livability (%):				
Control (fish diet)	93.5±17	-	-	-
Raw	-	98.3±1.7	98.3±1.7	100.0
Autoclaved	-	93.5±1.7	93.5±1.7	100.0
Raw + 0.5 g/kg enzyme	-	93.5±1.7	93.5±1.7	100.0
Raw + 1.0 g/kg enzyme	-	98.3±1.7	93.5±1.7	98.3±1.7
Raw + 2.0 g/kg enzyme	-	100.0	93.5±3.5	98.3±1.7

± Standard error

Analysis of variance

Source of variance	df	MSS
Treatments	15	107.86
Error	32	134.92

4.2.2.1 Experiment 3: Utilization of raw squilla meal in layers

The interaction response between levels of squilla meal in diet and laying periods for any of the parameter in experiment 3 is not significant.

4.2.2.1.1 Hen-day egg production: The differences due to levels of squilla meal in diet and due to periods in hen-day egg production were significant ($P<0.01$). Inclusion of squilla meal at 44.5 and 88.9 g/kg at the expense of fish protein had no significant effect ($P>0.05$) on hen-day egg production. However, hen-day egg production at 133.4 g/kg level was significantly ($P<0.01$) lower (72.8%) than on control (78.7%) and as well as at 44.5 g squilla meal per kg but was comparable to that on at 88.9 g squilla meal per kg diet (Table 26 and Fig.6).

4.2.2.1.2 Feed consumption and feed efficiency: There were no significant differences in the feed intake values observed on different dietary treatments (Table 27). The feed intake on diet varying in squila meal ranged from 111 to 115 g/b/d and these differences were not significant. The feed intake values observed in laying periods differed significantly ($P<0.05$) from each other.

Dietary treatments and as well as period had a significant ($P<0.05$) influence on feed conversion ratio

Table 26: Effect of varying dietary levels of squilla meal on hen-day egg production in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Hen-day egg production, % Periods 1	2	3	4	Mean ± SE
0 (Fish control)	72.8	78.8	83.9	79.1	$78.7^{ab}\pm1.4$
44.5	76.0	79.2	83.3	78.8	$79.3^{a}\pm1.0$
88.9	70.6	74.0	80.0	74.4	$74.8^{bc}\pm1.8$
133.4	63.9	70.2	83.3	73.8	$72.8^{c}\pm1.7$
Mean±SE	70.8^{z} ±1.5	75.0^{y} ±1.6	82.6^{x} ±1.3	76.5^{y} ±1.3	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	148.50**
Due to periods	3	421.94**
Treatments x Periods	9	24.37
Error	128	32.44

** Significant (P<0.01)

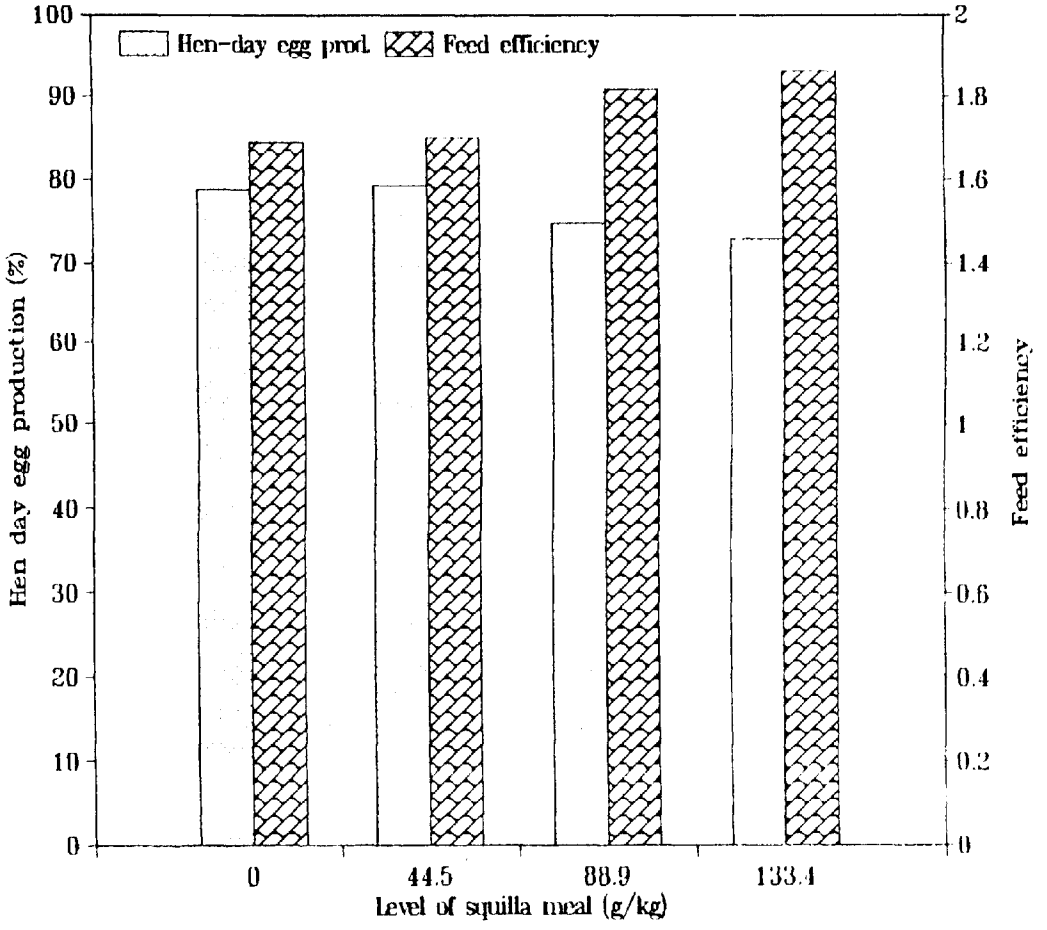

Fig.6. Hen-day egg production and feed efficiency of WL layers (30-45 wk) on various levels of squilla meal

Table 27: Effect of varying dietary levels of squilla meal on feed consumption of WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Feed consumption, g/hen/day — Periods 1	2	3	4	Mean ± SE
0 (Fish control)	90	118	122	114	111 ± 4
44.5	92	121	122	114	112 ± 4
88.9	89	122	128	122	115 ± 5
133.4	83	118	126	122	112 ± 5
Mean±SE	88^{a}±1	120^{bc}±2	125^{c}±2	118^{b}±3	

± Standard error

Means in a row bearing a common superscript are not significantly (P>0.05) different

Analysis of variance

Source of variance	df	MSS
Between treatments	3	37.24
Due to periods	3	3246.24**
Treatments x Periods	9	38.95
Error	32	46.13

** Significant (P<0.01)

values (Table 28 and Fig.6). Feed efficiency values obtained with reference and squilla meal at 44.5 g/kg were similar. However, increasing the level of squilla meal to 88.9 and 133.4 g/kg resulted in lowered ($P<0.05$) feed efficiency than control but statistically comparable to each other.

4.2.2.1.3 Egg weight: The egg weights on reference and squilla meal at 44.5 and 88.9 g/kg diets were similar (Table 29) and significantly ($P<0.01$) higher than that on diet containing squilla meal at 133.4 g/kg. The differences in egg weight observed between periods were significant ($P<0.01$).

4.2.2.1.4 Haugh unit score: The Haugh unit scores were also influenced both by treatment as well as periods (Table 30). Inclusion of squilla meal at 88.9 g/kg replacing fish protein gave slightly higher Haugh unit scores than on control diet and diets having squilla meal at other levels.

4.2.2.1.5 Albumen index: Albumen index (Table 31) was influenced significantly ($P<0.01$) both by dietary regimen as well as periods. Value on diet with squilla meal at 133.4 g/kg was significantly ($P<0.01$) higher than those on other diets.

4.2.2.1.6 Yolk index: Treatment and period differences were observed in yolk index values (Table 32). Inclusion of squilla meal at the expense of fish protein at 44.5 and 88.9

Table 28: Effect of varying dietary levels of squilla meal on efficiency of feed utilization of WL layers (30-45 wk, experiment 3).

Squilla meal, g/kg	Feed, kg/dozen eggs — Periods 1	2	3	4	Mean ± SE
0 (Fish control)	1.47	1.81	1.75	1.74	1.69^{a}±0.04
44.5	1.45	1.83	1.75	1.74	1.70^{a}±0.06
88.9	1.52	1.95	1.86	1.96	1.82^{b}±0.07
133.4	1.55	2.06	1.83	1.99	1.86^{b}±0.06
Mean±SE	1.50^{x} ±0.03	1.91^{y} ±0.04	1.86^{y} ±0.05	1.80^{y} ±0.05	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	0.0896*
Due to periods	3	0.4082*
Treatments x Periods	9	0.0008
Error	32	0.0186

* : Significant (P>0.05)

Table 29: Effect of varying dietary levels of squilla meal on egg weight in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Egg weight (g) Periods 1	2	3	4	Mean ± SE
0 (Fish control)	50.3	51.5	52.4	53.7	$52.0^{a}\pm0.3$
44.5	50.2	51.2	53.5	54.0	$52.2^{a}\pm0.3$
88.9	48.3	50.7	53.6	55.8	$52.1^{a}\pm0.5$
133.4	48.1	50.1	51.1	52.2	$50.4^{b}\pm0.4$
Mean±SE	49.2^{z} ±0.3	50.9^{yz} ±0.3	52.6^{y} ±0.4	53.9^{x} ±0.3	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	82.59**
Due to periods	3	458.52**
Treatments x Periods	9	20.63
Error	416	10.72

** Significant (P<0.01)

Table 30: Effect of varying dietary levels of squilla meal on Haugh unit score in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Haugh unit score — Periods 1	2	3	4	Mean ± SE
0 (Fish control)	93	91	90	85	$90^{b}\pm0.8$
44.5	95	95	91	87	$92^{a}\pm0.6$
88.9	93	90	90	86	$91^{ab}\pm0.6$
133.4	94	93	92	88	$92^{a}\pm0.7$
Mean±SE	$94^{x}\pm0.7$	$92^{y}\pm0.6$	$91^{y}\pm0.7$	$87^{z}\pm0.7$	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	138.96*
Due to periods	3	882.43**
Treatments x Periods	9	37.74
Error	416	46.13

* Significant (P<0.05); ** Significant (P<0.01)

Table 31: Effect of varying dietary levels of squilla meal on Albumen Index in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Albumen Index (Periods 1	2	3	4)	Mean ± SE
0 (Fish control)	0.132	0.116	0.118	0.097	0.116^{b}±0.003
44.5	0.135	0.122	0.119	0.102	0.120^{b}±0.002
88.9	0.136	0.115	0.122	0.110	0.121^{b}±0.002
133.4	0.156	0.126	0.125	0.111	0.130^{a}±0.003
Mean±SE	0.140^{x} ±0.002	0.120^{y} ±0.002	0.121^{y} ±0.003	0.105^{z} ±0.002	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	0.003635**
Due to periods	3	0.021591**
Treatments x Periods	9	0.000585
Error	416	0.000594

** Significant (P<0.01)

Table 32: Effect of varying dietary levels of squilla meal on yolk index in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Yolk index — Periods 1	2	3	4	Mean ± SE
0 (Fish control)	0.446	0.434	0.440	0.399	0.431^{b}±0.002
44.5	0.439	0.443	0.434	0.396	0.429^{b}±0.003
88.9	0.446	0.439	0.432	0.411	0.433^{b}±0.002
133.4	0.465	0.446	0.445	0.411	0.442^{a}±0.003
Mean±SE	0.449^{X} ±0.003	0.441^{Y} ±0.002	0.438^{Y} ±0.003	0.405^{Z} ±0.002	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	0.003835**
Due to periods	3	0.041006**
Treatments x Periods	9	0.000829
Error	416	0.000599

** Significant (P<0.01)

g/kg levels gave yolk index values (0.429 and 0.433) which are comparable with that on control (0.431). Squilla meal inclusion at 133.4 g/kg in diet resulted in significantly ($P<0.01$) high yolk index value (0.442). Like Haugh unit score and albumen index, the yolk index values were also found to be lower with advancing age of the birds.

4.2.2.1.7 Egg shell thickness: The egg shell thickness of hens fed on different levels of squilla meal was not significantly ($P>0.05$) different from that on control (Table 33), but significant ($P<0.05$) period differences were observed.

4.2.2.2 Experiment 4: Utilization of raw and autocalved squilla meal and raw squilla meal supplemented with enzyme in layers

The interaction between level of squilla meal and the period by which measurements were taken was significant ($P<0.01$) only for albumen index, Haugh unit score and yolk index values.

4.2.2.2.1 Hen-day egg production: Significant ($P<0.05$) differences existed in hen-day egg production due to different dietary treatments and periods (Table 34 and Fig.7). At 88.9 g/kg in layer diet, raw and autoclaved squilla meal or raw squilla supplemented with enzyme at 0.5 to 2.0 g/kg diet did not influence the hen-day egg production.

Table 33: Effect of varying dietary levels of raw squilla meal on shell thickness in WL layers (30-45 wk, experiment 3)

Squilla meal, g/kg	Shell thickness (mm) Periods 1	2	3	4	Mean ± SE
0 (control)	0.355	0.361	0.357	0.364	0.359±0.002
44.5	0.364	0.367	0.362	0.363	0.364±0.002
88.9	0.364	0.366	0.358	0.364	0.363±0.001
133.4	0.362	0.367	0.357	0.363	0.362±0.002
Mean±SE	0.361^{b} ±0.001	0.365^{a} ±0.002	0.359^{b} ±0.002	0.363^{b} ±0.001	

± Standard error

Means in a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	3	0.000433
Due to periods	3	0.000927*
Treatments x Periods	9	0.000151
Error	416	0.000237

* Significant (P<0.05)

Table 34: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on hen-day egg production in WL layers (25-40 wk, experiment 4)

Diet	Hen-day egg production, %				Mean ± SE
	Periods				
	1	2	3	4	
Control (Fish diet)	90.2	96.9	93.1	92.3	$93.1^{a}\pm0.9$
Squilla meal, 88.9 g/kg replacement:					
Raw	90.2	96.7	93.8	91.1	$92.9^{a}\pm0.9$
Autocalaved	86.3	94.3	93.7	89.6	$91.0^{abc}\pm1.0$
Raw + enzyme premix 0.5 g/kg diet	88.4	97.3	92.9	91.1	$92.4^{ab}\pm0.9$
Raw + enzyme premix 1.0 g/kg diet	89.0	97.0	94.3	92.3	$93.1^{a}\pm0.9$
Raw + enzyme premix 1.0 g/kg diet	85.1	98.2	92.6	90.5	$91.6^{ab}\pm1.3$
Squilla meal, 133.4 g/kg replacement:					
Raw	87.8	91.7	88.7	86.9	$88.8^{c}\pm0.8$
Autoclaved	86.6	90.5	90.2	87.8	$88.8^{c}\pm0.8$
Raw + enzyme premix 0.5 g/kg diet	87.8	96.4	93.5	92.6	$92.6^{ab}\pm1.0$
Raw + enzyme premix 1.0 g/kg diet	87.5	96.1	94.3	91.7	$92.4^{ab}\pm0.9$
Raw + enzyme premix 2.0 g/kg diet	86.6	95.8	90.1	89.0	$90.4^{bc}\pm1.6$
Mean±SE	87.8^{z} ±0.7	95.5^{w} ±0.4	92.5^{x} ±0.5	90.4^{y} ±0.5	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.01)

Analysis of variance

Source of variance	df	MSS
Between treatments	10	63.90**
Due to periods	3	711.54**
Treatments x Periods	30	10.13
Error	220	17.96

** Significant (P<0.01)

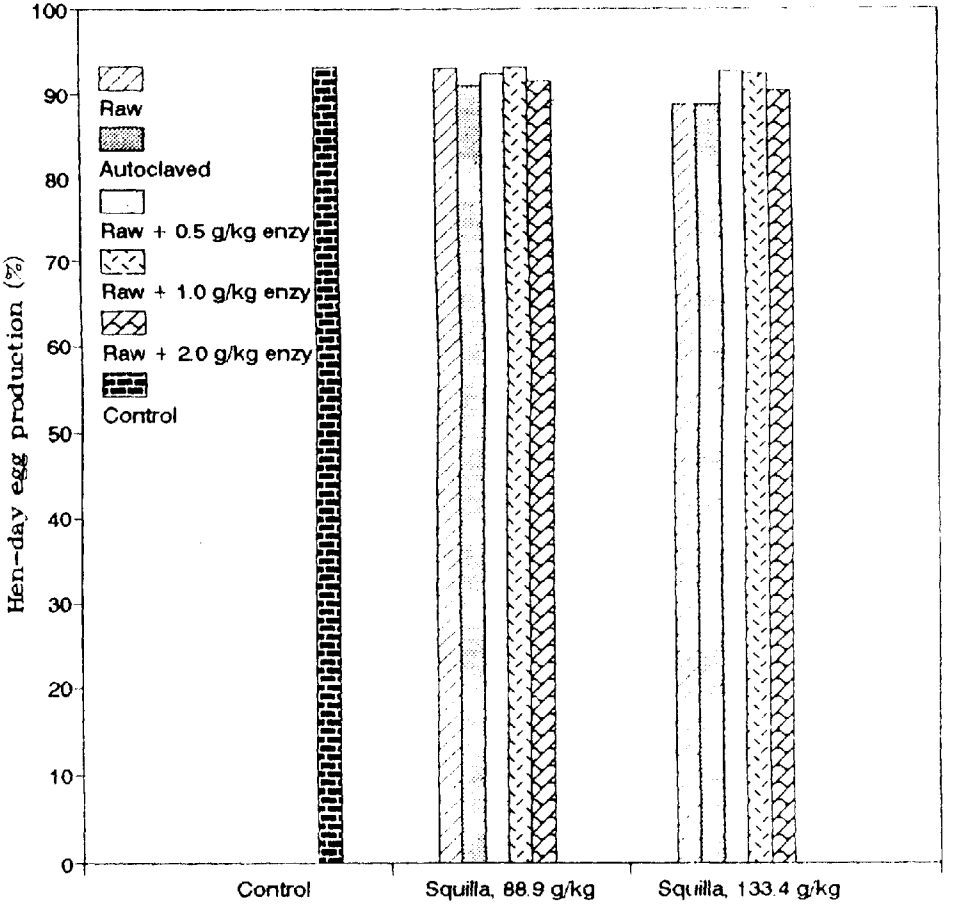

Fig.7: Hen-day egg production of WL layers on variously treated squilla meal

However, the same test materials raw and autoclaved squilla meal at 133.4 g/kg levels replacing complete fish protein resulted in significantly ($P<0.01$) decreased egg production than on control. Enzyme supplementation at 0.5 and 1.0 g/kg improved egg production compared to that on raw and autoclaved squilla meal. The egg production on 0.5 and 1.0 g/kg was similar to that on reference diet but that on 2.0 g/kg was significantly ($P<0.01$) lower.

4.2.2.2.2 Feed consumption and feed efficiency: The feed intake values (Table 35) on different diets and for different periods were not significantly ($P>0.05$) different. Feed intake on raw squilla meal at both inclusion levels (88.9 and 133.4 g/kg) was almost similar (119 and 120 g/bird/day). Feed intake on autoclaved squilla meal was higher (122 and 120 g) on both levels (88.9 and 133.4 g/kg) than control (117.0 g). Feed intake on squilla meal at 88.9 g/kg level with 2.0 g/kg enzyme supplementation was also higher (122.0 g).

Dietary treatments and periods had significantly ($P<0.05$) influenced feed conversion ratio values (Table 36 and Fig.8). Feed efficiency obtained on autoclaved squilla meal at both levels (88.9 and 133.4 g/kg) and raw squilla meal at 133.4 g/kg level was similar (1.62) and was significantly ($P<0.05$) poorer than on control (1.51).

Table 35: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla supplemented with enzyme on feed consumption in WL layers (25-40 wk, experiment 4)

Diet	Feed consumption, g/hen/dat				Mean ± SE
	Periods				
	1	2	3	4	
Control (Fish diet)	113	116	120	120	117±1.6
Squilla meal, 88.9 g/kg replacement:					
Raw	120	116	118	122	119±1.4
Autoclaved	118	117	127	127	122±2.0
Raw + enzyme premix 0.5 g/kg diet	116	121	118	121	119±1.4
Raw + enzyme premix 1.0 g/kg diet	120	116	110	118	115±1.6
Raw + enzyme premix 2.0 g/kg diet	122	121	117	126	122±1.7
Squilla meal, 133.4 g/kg replacement:					
Raw	122	119	115	123	120±2.7
Autoclaved	121	120	114	123	120±1.5
Raw + enzyme premix 0.5 g/kg diet	112	120	118	122	118±2.4
Raw + enzyme premix 1.0 g/kg diet	123	114	120	125	121±2.1
Raw + enzyme premix 2.0 g/kg diet	119	120	114	123	120±1.5
Mean±SE	119 ±1.4	118 ±0.8	118 ±1.0	123 ±1.0	

± Standard error

Analysis of variance

Source of variance	df	MSS
Between treatments	10	41.20
Due to periods	3	180.25
Treatments x Periods	30	32.33
Error	88	39.12

Table 36: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on efficiency of feed utilization in WL layers (25 40 wk, experiment 4)

Diet	Feed, kg/dozen eggs, Periods 1	2	3	4	Mean ± SE
Control (Fish diet)	1.50	1.43	1.55	1.56	$1.51^{a}\pm0.02$
Squilla meal, 88.9 g/kg replacement:					
Raw	1.60	1.44	1.51	1.61	$1.54^{abc}\pm0.03$
Autoclaved	1.63	1.49	1.63	1.72	$1.62^{c}\pm0.03$
Raw + enzyme premix 0.5 g/kg diet	1.57	1.50	1.52	1.60	$1.55^{abc}\pm0.02$
Raw + enzyme premix 1.0 g/kg diet	1.62	1.43	1.40	1.53	$1.50^{a}\pm0.03$
Raw + enzyme premix 2.0 g/kg diet	1.72	1.52	1.52	1.67	$1.61^{bc}\pm0.03$
Squilla meal, 133.4 g/kg replacement:					
Raw	1.66	1.58	1.55	1.68	$1.62^{c}\pm0.04$
Autoclaved	1.68	1.61	1.50	1.68	$1.62^{c}\pm0.03$
Raw + enzyme premix 0.5 g/kg diet	1.53	1.49	1.51	1.58	$1.53^{ab}\pm0.03$
Raw + enzyme premix 1.0 g/kg diet	1.69	1.42	1.53	1.63	$1.57^{abc}\pm0.04$
Raw + enzyme premix 2.0 g/kg diet	1.67	1.50	1.58	1.68	$1.61^{bc}\pm0.04$
Mean±SE	1.62^{y} ±0.02	1.49^{x} ±0.01	1.63^{y} ±0.02	1.53^{x} ±0.01	

± Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	10	0.0272*
Due to periods	3	0.1641*
Treatments x Periods	30	0.0066
Error	88	0.0088

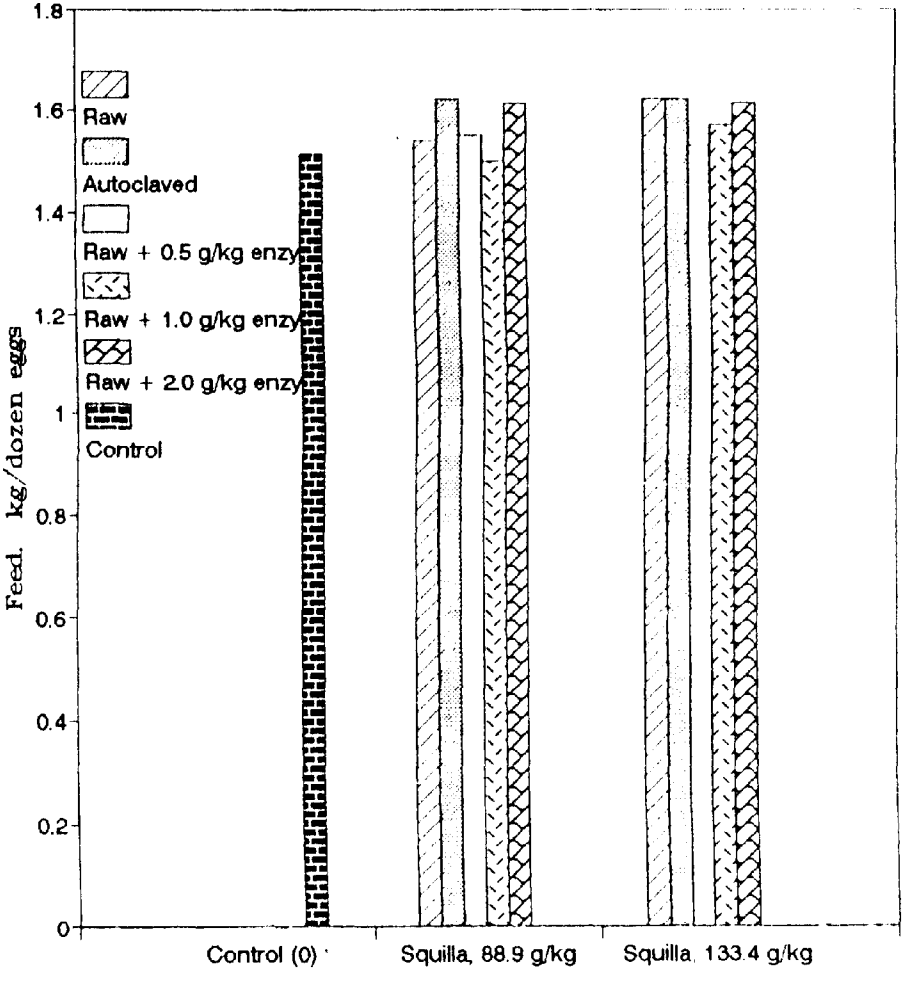

Fig.8: Feed efficiency of WL layers (25–40 wk) on variously treated squilla meal

Enzyme supplementation tended to improve feed efficiency at 0.5 and 1.0 g/kg diet. Surprisingly, the highest enzyme supplementation level (2.0 g/kg) caused significantly ($P<0.05$) lowered feed efficiency for both squilla meal diets.

4.2.2.2.3 Egg weight: The egg weight values obtained on different diets were significantly ($P<0.05$) different from one another (Table 37). Period differences were also significant ($P<0.01$). Egg weight on raw, autoclaved and enzyme supplemented (1.0 g/kg) squilla meal at 133.4 g/kg levels, were significantly ($P<0.05$) lower than on control. Supplementation of enzyme premix at 0.5 and 2.0 g/kg to the raw squilla meal at 133.4 g/kg level improved the egg weight (53.4 and 53.1 g) and these were comparable to that on control (53.7 g). Egg weight obtained on raw, autoclaved and enzyme supplemented squilla meal at 88.9 g/kg level were comparable to that on control. However, enzyme supplemented (1.0 g/kg) to 133.4 g/kg squilla meal resulted in a significantly ($P<0.05$) lower egg weight.

4.2.2.2.4 Haugh unit score: Haugh unit scores were influenced ($P<0.01$) by dietary treatments as well as by treatment x period interaction (Table 38). At 88.9 g/kg raw and autoclaved squilla meal and raw squilla supplemented with enzyme at 1 g/kg diet resulted in lowered Haugh unit scores than on control. However, at 133.4 g/kg squilla meal, raw squilla meal supplemented with enzyme at 0.5 and

Table 37: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on egg weight in WL layers (25-40 wk, experiment 4)

Diet	Egg weight, (g) Periods 1	2	3	4	Mean ± SE
Control (Fish diet)	50.9	52.7	55.1	56.2	$53.7^{a}\pm0.5$
Squilla meal, 88.9 g/kg replacement:					
Raw	51.4	52.4	52.9	54.1	$52.7^{bcde}\pm0.4$
Autoclaved	51.3	53.3	54.5	55.2	$53.6^{ab}\pm0.4$
Raw + enzyme premix 0.5 g/kg diet	50.4	51.2	53.1	55.0	$52.6^{cde}\pm0.4$
Raw + enzyme premix 1.0 g/kg diet	50.3	51.9	54.0	54.9	$52.8^{abcde}\pm0.3$
RAw + enzyme premix 2.0 g/kg diet	51.3	53.8	53.2	56.4	$53.7^{a}\pm0.4$
Squilla meal, 133.4 g/kg replacement:					
Raw	51.1	52.6	51.6	53.0	$52.0^{e}\pm0.4$
Autoclaved	50.5	52.1	54.2	53.2	$52.5^{cde}\pm0.3$
Raw + enzyme premix 0.5 g/kg diet	50.5	52.5	54.0	56.5	$53.4^{abc}\pm0.4$
Raw + enzyme premix 1.0 g/kg diet	50.1	51.8	52.9	54.3	$52.2^{de}\pm0.4$
Raw + enzyme premix 2.0 g/kg diet	50.5	52.7	53.9	54.5	$53.1^{abcd}\pm0.3$
Mean±SE	50.8^{z} ±0.2	52.6^{y} ±0.2	53.6^{x} ±0.2	54.9^{w} ±0.2	

±Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	10	25.11*
Due to periods	3	607.50**
Treatments x Periods	30	9.98
Error	748	8.96

* Significant (P<0.05); ** Significant (P<0.01)

Table 38: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla supplemented with enzyme on haugh unit score in WL layers (25-40 wk, experiment 4)

Diet	Haugh unit score (Periods 1	2	3	4)	Mean ± SE
Control (Fish diet)	85	87	87	86	$86^{d}\pm0.8$
Squilla meal, 88.9 g/kg replacement:					
Raw	84	85	84	88	$85^{cd}\pm0.7$
Autoclaved	80	82	83	83	$82^{a}\pm0.6$
Raw + enzyme premix 0.5 g/kg diet	83	87	85	85	$85^{cd}\pm0.7$
Raw + enzyme premix 1.0 g/kg diet	83	84	79	82	$82^{a}\pm0.7$
Raw + enzyme premix 2.0 g/kg diet	88	85	82	83	$85^{cd}\pm0.8$
Squilla meal, 133.4 g/kg replacement:					
Raw	81	83	86	87	$84^{bc}\pm0.7$
Autoclaved	84	86	85	87	$84^{bc}\pm0.4$
Raw + enzyme premix 0.5 g/kg diet	84	86	85	85	$85^{cd}\pm0.6$
Raw + enzyme premix 1.0 g/kg diet	82	83	83	83	$83^{ab}\pm0.9$
Raw + enzyme premix 2.0 g/kg diet	86	87	84	82	$86^{d}\pm0.6$
Mean±SE	84± 0.4	85± 0.4	84± 0.4	85± 0.4	

± Standard error

Means in a column bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	10	178.58**
Due to periods	3	110.27
Treatments x Periods	30	49.17**
Error	748	14.30

** Significant (P<0.01)

2.0 g/kg, resulted in comparable Haugh unit scores with that on control.

4.2.2.2.5 Albumen index: Albumen index was influenced significantly ($P<0.01$) among dietary treatments, period and treatment x period interaction (Table 39). The influence of squilla meal in diet on albumen index had not shown any specific trend. The albumen index values obtained with autoclaved squilla meal at 88.9 g/kg and squilla meal with enzyme supplementation at (1.0 and 2.0 g/kg) levels were significantly lower. Nevertheless, albumen index values on raw, autoclaved squilla meal and raw squilla meal supplemented with enzyme at 0.5 and 2.0 g/kg diet contained squilla meal at 133.4 g/kg were not significantly ($P>0.05$) different.

4.2.2.2.6 Yolk index: Yolk index values were influenced by treatments, periods and treatment x period interaction (Table 40). The diet containing squilla at 133.4 g/kg supplemented with enzyme at 2.0 g/kg resulted in lowered ($P<0.05$) yolk index values than control diet.

4.2.2.2.7 Egg shell thickness: Egg shell thickness values (Table 41) were influenced by treatments and periods as well. On different levels of squilla, shell thickness values did not follow any specific trend.

Table 39: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on albumen index in WL layers (25-40 wk, experiment 4)

Diet	Albumen Index				Mean ± SE
	Periods				
	1	2	3	4	
Control (Fish diet)	0.106	0.105	0.101	0.096	$0.102^{abc}\pm0.002$
Squilla meal, 88.9 g/kg replacement:					
Raw	0.105	0.106	0.101	0.102	$0.104^{ab}\pm0.002$
Autoclaved	0.097	0.097	0.094	0.087	$0.094^{d}\pm0.002$
Raw + enzyme premix 0.5 g/kg diet	0.106	0.112	0.101	0.100	$0.104^{ab}\pm0.002$
Raw + enzyme premix 1.0 g/kg diet	0.105	0.103	0.082	0.085	$0.093^{d}\pm0.002$
Raw + enzyme premix 2.0 g/kg diet	0.116	0.107	0.088	0.088	$0.099^{bcd}\pm0.002$
Squilla meal, 133.4 g/kg replacement:					
Raw	0.103	0.106	0.111	0.098	$0.104^{ab}\pm0.002$
Autoclaved	0.105	0.111	0.096	0.101	$0.103^{ab}\pm0.002$
Raw + enzyme premix 0.5 g/kg diet	0.107	0.111	0.103	0.096	$0.104^{ab}\pm0.002$
Raw + enzyme premix 1.0 g/kg diet	0.101	0.100	0.093	0.091	$0.096^{cd}\pm0.002$
Raw + enzyme premix 2.0 g/kg diet	0.115	0.109	0.099	0.100	$0.106^{a}\pm0.002$
Mean±SE	0.106^{x} ±0.001	0.106^{x} ±0.001	0.097^{y} ±0.002	0.095^{y} ±0.001	

±Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of variance

Source of variance	df	MSS
Between treatments	10	0.00159**
Due to periods	3	0.03333**
Treatments x Periods	30	0.16666**
Error	748	0.00034

** Significant (P<0.01)

Table 40: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on yolk index in WL layers (25-40 wk, experiment 4)

Diet	Yolk Index (Periods 1	2	3	4)	Mean ± SE
Control (Fish diet)	0.440	0.449	0.435	0.442	$0.442^{ab}\pm0.003$
Squilla meal, 88.9 g/kg replacement:					
Raw	0.443	0.445	0.432	0.434	$0.439^{a}\pm0.002$
Autoclaved	0.470	0.465	0.426	0.437	$0.450^{bcd}\pm0.003$
Raw + enzyme premix 0.5 g/kg diet	0.453	0.455	0.444	0.442	$0.449^{bcd}\pm0.003$
Raw + enzyme premix 1.0 g/kg diet	0.455	0.451	0.442	0.433	$0.445^{abc}\pm0.003$
Raw + enzyme premix 2.0 g/kg diet	0.461	0.458	0.435	0.429	$0.446^{abcd}\pm0.003$
Squilla meal, 133.4 g/kg replacement:					
Raw	0.450	0.454	0.445	0.432	$0.446^{abcd}\pm0.003$
Autoclaved	0.445	0.457	0.436	0.437	$0.444^{ab}\pm0.003$
Raw + enzyme premix 0.5 g/kg diet	0.447	0.457	0.436	0.434	$0.444^{ab}\pm0.003$
Raw + enzyme premix 1.0 g/kg diet	0.446	0.450	0.419	0.435	$0.440^{a}\pm0.004$
Raw + enzyme premix 2.0 g/kg diet	0.474	0.461	0.445	0.434	$0.454^{d}\pm0.003$
Mean±SE	0.453^{y} ±0.002	0.455^{y} ±0.002	0.437^{x} ±0.002	0.436^{x} ±0.001	

±Standard error

Means in a column or a row bearing a common superscript are not significantly different (P>0.05)

Analysis of Variance

Source of variance	df	MSS
Between treatments	10	0.00150*
Due to periods	3	0.02130**
Treatments x Periods	30	0.00093**
Error	128	0.00056

* Significant (P<0.05); ** Significant (P<0.01)

Table 41: Effect of varying dietary levels of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme on shell thickness in WL layers (25-40 wk, experiment 4)

Diet	Shell thickness (mm) Periods 1	2	3	4	Mean ± SE
Control (Fish diet)	0.378	0.374	0.375	0.374	$0.375^{abc}\pm0.0019$
Squilla meal, 88.9 g/kg replacement:					
Raw	0.369	0.374	0.378	0.373	$0.374^{ab}\pm0.0023$
Autoclaved	0.363	0.381	0.377	0.369	$0.373^{ab}\pm0.0023$
Raw + enzyme premix 0.5 g/kg diet	0.390	0.383	0.384	0.376	$0.384^{d}\pm0.0018$
Raw + enzyme premix 1.0 g/kg diet	0.377	0.377	0.323	0.378	$0.379^{bcd}\pm0.0018$
Raw + enzyme premix 2.0 g/kg diet	0.377	0.376	0.381	0.366	$0.375^{abc}\pm0.0022$
Squilla meal, 133.4 g/replacement:					
Raw	0.363	0.373	0.378	0.367	$0.370^{a}\pm0.0024$
Autoclaved	0.368	0.383	0.373	0.367	$0.373^{ab}\pm0.0025$
Raw + enzyme premix 0.5 g/kg diet	0.379	0.386	0.392	0.363	$0.380^{cd}\pm0.0024$
Raw + enzyme premix 1.0 g/kg diet	0.378	0.379	0.386	0.365	$0.377^{bcd}\pm0.0023$
Raw + enzyme premix 2.0 g/kg diet	0.379	0.386	0.389	0.365	$0.380^{cd}\pm0.0019$
Mean±SE	0.375^{b} ±0.0013	0.379^{b} ±0.0013	0.382^{c} ±0.0015	0.369^{x} ±0.0011	

± Standard error
Means in a column or row bearing common superscript are not significantly different (P>0.05)

Analysis of Variance

Source of variance	df	MSS
Between treatments	10	0.001134*
Due to periods	3	0.005682**
Treatments x Periods	30	0.000507
Error	748	0.000316

* Significant (P<0.05); ** Significant (P<0.01)

CHAPTER V

DISCUSSION

The findings of investigations on the nutritive value of squilla meal in comparison to that of fish meal in part-I and the utilization of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme for poultry in Part II are discussed.

PART - I

5.1 NUTRITIVE VALUE OF SQUILLA

5.1.1 *In vitro* studies

5.1.1.1 Proximate principles and trace mineral composition

Squilla meal, on dry matter basis, had 339.7 g protein and 18.6 g ether extract per kg representing 62 per cent protein and 57 per cent ether extract of that present in fish meal (Table 14). Madhavan and Nair (1975) reported higher values of protein (447.1 g/kg) and ether extract (26.8 g/kg) in squilla meal. Mathew *et al.* (1982) in protein extract of squilla meal and Lekshmy Nair *et al.* (1991) in squilla protein powder reported higher values of protein and ether extract than those observed in these studies.

The crude fibre content of squilla meal (139.2 g/kg) was higher than that of fish meal (32.0 g/kg). It was

expected, that squilla meal being a crustacean, to have higher fibre mostly in the form of chitin than in fish meal. A chitin content of 147 g/kg in squilla meal (Madhavan and Nair, 1975) and 129 g/kg in crab meal (Lubitz *et al.*, 1943) was reported. Squilla meal contained total ash content of 383.1 g/kg, a value higher than in fish meal (314.5 g/kg). Both the squilla meal and fish meal employed were comparable in their content of calcium and phosphorus squilla meal however, had more of magnesium, zinc and iron than fish meal. The total ash content obtained in this study is in accordance with the values reported by Madhavan and Nair (1975).

5.1.1.3 Amino acid profile

The content of all the amino acids analysed, except that of tyrosine was lower in squilla meal than in fish meal. The critical amino acids for poultry feeding i.e., lysine, arginine, threonine, methionine and tryptophan were much lower in squilla meal ranging from 43 to 79 per cent of that in fish meal (Table 15).

5.1.2 *In vivo* studies

Protein quality of squilla meal assessed as GPV and protein digestibility and its metabolizable energy values determined in three separate experiments are discussed below.

5.1.2.1 Gross protein value

The marginal differences in the GPV values of squilla and fish (68 vs 72%) may be attributed to the similar marginal differences observed in the total protein consumption 30.76 vs 31.97 g/bird (Table 16). It is also evident from the data of Rand *et al.* (1960) where the growth responses were proportional to protein intake. Similar, conclusions have also been drawn by Heiman *et al.* (1939). This can be further justified from the fact that protein quality of squilla meal in terms of its amino acid profile was also lower than fish meal.

5.1.2.2 Protein digestibility or absorbability:

Comparatively lower (66.0%) protein digestibility observed with squilla meal than with fish meal (74.0%) may be due to a higher fibre (chitin) content in squilla meal than in fish meal. Lodhi *et al.* (1970) reported that the lower digestibility in rapeseed than soyabean meal may be due to high fibre and low protein of that feedstuff. High fibre diets were shown to reduce digestibility of protein (Kratzer *et al.*, 1967). Similar observations were made by Rajasekhar Reddy *et al.* (1989) in Ambadi cake in comparison to groundnut cake. Nevertheless Hirano *et al.* (1990) has indicated very high digestibility (88-98%) of pure chitin in broilers.

5.1.2.3 Metabolizable energy

Fish meal and squilla meal used in the experiments were low in energy. Fish meal had AMEn of 8.08 MJ/kg while squilla meal had still a lower value of 7.13 MJ/kg. Low ether extract and high ash in fish meal are responsible for its low ME content. Squilla meal contained slightly lower ether extract and high ash and much higher crude fibre than fish meal. Thus squilla meal contained low content of ME than fish meal.

PART - II

5.2 UTILIZATION OF SQUILLA MEAL IN CHICKEN

The data of results on the utilization of squilla meal for growth and egg production in chicken have been discussed in this section.

5.2.1 Utilization of squilla meal for growth (Experiment 1 and 2)

Results obtained on inclusion of squilla meal replacing fish mixture at four levels (41.7, 83.4, 125.1 and 166.8 g/kg) in broiler diet (Experiment 1) and the inclusion of raw and autoclaved squilla meal (83.4, 125.1 and 166.8 g/kg) and supplementation of enzyme premix (0.5, 1.0 and 2.0 g/kg diet) to the above three levels of raw squilla meal in the broiler diet (Experiment 2) are discussed in this section.

5.2.1.1 Experiment 1: utilization of raw squilla meal for broiler (1-42 d,age).

5.2.1.1.1 Body weight gain: Inclusion of raw squilla meal at different dietary levels (41.7, 83.4, 125.1 and 166.8 g/kg) replacing fish protein in control diet caused no significant (P>0.05) differences in body weight gains (Table 19). The appreciably lowered body weight gain on diet having the highest squilla meal content (166.8 g/kg) than on control diet may probably be due to the contributions of lower levels of certain critical amino acids by squilla meal and/or lower digestibility of squilla meal protein and higher chitin content of squilla meal. Table 42 shows that lysine, arginine, threonine and methionine progressively became lower in diet with increasing the level of squilla meal. Expressing amino acid concentration in relation to ME content of diet, arginine, lysine, and methionine became deficient with squilla meal at and above 83.4 g/kg diet and threonine at and above 125.1 g/kg diet. Squilla meal in our studies had 66 per cent protein digestibility, while fish meal had 74 per cent. Assuming the digestibility of protein to the digestibility of amino acids, contributions of digestible critical amino acids from fish meal and squilla meal are shown in Table 42. This also indicates the possibility that the lowered contributions of lysine, arginine, threonine and methionine might have been accentuated by the lower digestibility of squilla protein.

Table 42: Essential amino acid composition (as such basis) of broiler diets containing varying levels of squilla meal (Experiment 1 & 2).

Essential amino acid (%)	Fish based control diet	Fish protein replaced by squilla meal at (%)			
		25	50	75	100
Arginine	1.14	1.10	1.06	1.02	0.98
Glycine plus serine	1.70	1.68	1.66	1.65	1.63
Histidine	0.45	0.45	0.44	0.44	0.44
Isoleucine	0.77	0.76	0.75	0.75	0.74
Lysine	1.02	0.99	0.95	0.91	0.87
Leucine	1.63	1.61	1.59	1.57	1.55
Methionine	0.45	0.44	0.43	0.42	0.41
Phenylalanine	0.84	0.83	0.82	0.81	0.80
Tyrosine	0.45	0.45	0.45	0.45	0.45
Threonine	0.74	0.72	0.70	0.68	0.66
Tryptophan	0.46	0.46	0.46	0.45	0.45
Valine	0.87	0.86	0.86	0.85	0.84

Squilla meal contained 13.9 per cent chitin. Earlier findings support that chitin at lower levels (0.5%) is probably digested by the chitinase enzyme secreted by the intestinal bacteria. Moreover, the feed ingredients present in diet have chitinase activity (Hirano *et al.*, 1988). Besides, at low levels (0.5%) chitin is shown to have growth promoting effects by producing glucosamine from the hydrolysis of chitin caused by chitinolytic enzymes present in the chicken intestine (Ramachandran Nair *et al.*, 1987). Spreen *et al.* (1984) have also reported that chitinous materials in the intestinal tract of chicks supported the growth of *Bifidobacterium*, thus stimulating improved gains. A similar improved performance by inclusion of 5 per cent shrimp by-catch meal has also been reported by Ilian *et al.* (1985). In the present experiment, the chitin content of the diets was 0.58, 1.16, 1.74 and 2.32 per cent contributed by 4.17, 8.34, 12.51 and 16.68 per cent levels of squilla meal, respectively. Growth promoting effect was not much evident in the present experiment, except for the numerically higher (1372 g) gains on 4.17 per cent level of squilla meal than on control (1347 g). At the higher level of inclusion, chitin probably might have reduced growth as evidenced by the appreciable reduction in weight gains on squilla meal diet compared with other diets.

5.2.1.1.2 Feed consumption and feed efficiency: The isonitrogeneous and isoenergetic diets of varied levels of

squilla meal, generally showed increase in feed intake with increase of squilla meal in the diet. The increased feed intake probably, may be due to lowered levels of critical amino acids (lysine, threonine, methionine and arginine) and or higher level of chitin as discussed in section 5.2.1.1.1. The results obtained in this study, are in accordance with the results of Ramachandra Nair *et al.* (1987) who observed 5.0 per cent increase in feed intake at 5.0 g per kg level of chitin in the diet. In this study the increase in feed intake was to the extent of 8 to 10 per cent on diets containing 41.7 and 83.4 g per kg squilla meal contributing 5.8 and 11.6 g per kg chitin,respectively. However, when the chitin content increased further to 17.4 and 23.2 g per kg the increase in feed intake was not proportional but it was only 6 and 5 per cent,respectively.

Non-significance ($P>0.05$) effects were recorded in feed efficiency for all inclusion levels of squilla meal in broiler diets, indicated the potential of squilla meal to replace fish protein without adversely affecting performance. On the diet containing the highest level of squilla meal the lowered growth and higher feed intake might be responsible for appreciably lowered FE than on other diets.

Feed efficiency has been shown to improve by the inclusion of chitin to the broiler diets as reported by

various workers (Ramachandra Nair *et al.*, 1987; Narahari *et al.*, 1991; Ramachandra Nair *et al.*, 1993). However in this study the feed efficiency has not improved with the chitin/squilla meal levels but slightly decreased though non-significantly. The results of Kobayoshi and Itoh (1991) also revealed non significant differences in feed efficiency by the addition of 5 per cent chitin to broiler diets concur with the observations of this study.

5.2.1.1.3 Economics: The cost to produce one kg live weight was mostly influenced by the dietary intake. Similar cost of production of Rs.12.51 recorded on control and 125.1 g per kg squilla meal level diets indicated that squilla meal can be included at 125.1 g per kg replacing 75 per cent of fish nitrogen with comparable economy.

5.2.1.1.4 Weights of visceral organs : The statistically comparable weights of visceral organs viz. liver, spleen, kidney and pancreas obtained on different dietary treatments in this study, are in accordance with the one observed in rats by Lekshmy Nair *et al.* (1991). Regression analysis of data on weight of visceral organs (Liver, spleen, kidney and pancreas) did not reveal any significant linearity effect with varying levels of dietary squilla meal (Table 20).

5.2.1.1.5 Length of intestinal segments : The increase in length of intestinal segments in general with increasing inclusion levels of squilla meal in diets may probably due

to the increasing levels of chitin, which might have increased the digestion activity resulting in increased length of intestines. This is further supported by the regression analysis (Table 21) which indicated a significant linear effect of squilla meal on both duoderal and caecal lengths (P=0.0435 and P=0.0028 respectively).

The lower density of test diets may be another contributary factor. It has been demonstrated by Kondra *et al.* (1974) that chicken are capable of enlarging the length of digestive tract to obtain required nutrients from low density diets. Several other workers have also reported that high fibrous diets result in relatively increased length of intestine and caecum (Savory and Gentle., 1976; Abdelsami *et al.*, 1983) and Jejunum + ileum and caecum (Rajasekher *et al.*, 1993; Ravinder Reddy, 1993) due to higer feed intake.

5.2.1.2 Experiment 2: Utilization of raw and autoclaved squilla meal and raw squilla supplemented with enzyme in broilers (8-42 d age)

5.2.1.2.1 Body weight gain : Inclusion of raw squilla meal at 83.4 and 125.1 g per kg replacing fish meal N at 50 and 75 per cent of control diet caused no significant (P>0.05) depression of weight gains in broiler chicks (Table 22). It indicates that fish meal N can be replaced by squilla meal upto 125.1 g per kg (75 per cent) without any adverse

effect. However at 166.8 g per kg level of squilla meal weight gains observed were lower than on control. The trend in weight gains in this experiment is similar to that one observed in the earlier growth experiment, except that at 100 per cent replacement it reached the level of significance ($P<0.05$), while it was just numerically lower in earlier experiment. The causes for growth depression on higher level of squilla meal were already discussed in section 5.2.1.1.1.

Autoclaving of squilla meal did not improve the weight gains. Infact, it resulted in significantly ($P<0.05$) depressed body weight, at all inclusion levels than control. This might be attributed to the decreased availability of amino acids specifically lysine, and the inability of autoclaving to break down the chitin.

In order to improve the utility of squilla meal in poultry diets, chitinase enzyme supplementation was considered more appropriate. However, due to non-availability of this enzyme commercially, addition of bacterial proteases was considered as next most appropriate choice, Since the protein digestibility and quality in squilla meal is inferior to fish meal protein as evidenced by the GPV and protein digestibility values. The same view has been expressed by M/S Finn feeds International, U.K. (Personal communication).

Contrary to autoclaving, proteases enzyme supplementation gave favourable response. Significant improvement in weight gains for all the inclusion levels of squilla meal on 0.5 and 1 g/kg enzyme levels, when compared to the respective unsupplemented diets, might be due to improved utilization of protein because of proteolytic enzymes. Rexen (1981) have shown that enzyme supplementation increased weight gains than unsupplemented control. The effect was more pronounced when the feed comprised of less digestible feed ingredients. Similar observations have also been made by Kadam *et al.* (1991) and Padmanabhan (1992).

However, 2g per kg enzyme supplementation could not further enhance the performance, indicating the optimum level of supplementation of bacterial proteases enzyme premix was 0.5 to 1.0 g per kg of diet when complete fish meal 'N' is replaced by squilla meal 'N'. Since a similar and non-significant difference in performance was observed with both 0.5 and 1.0 g per kg enzyme levels; considering economic aspects, a lower level of enzyme @ 0.5 g/kg diet supplementation may be suggested as optimum. D 3308

5.2.1.2.2 Feed consumption and feed efficiency: The insignificant and non-specific trend of feed intake at varying levels of squilla meal irrespective of dietary treatments indicated that incorporation of squilla meal at the expense of fish nitrogen only caused numerical decrease in consumption levels (Table 23). The pattern of feed

intake on raw squilla meal diets at 83.4, 125.1 and 166.8 g per kg (50,75 and 100 per cent) replacement levels in this study is similar to one observed in earlier experiment, i.e. decreasing linearly with increasing squilla meal levels, which were still higher than control. Similar pattern was observed for autoclaved diets. This further confirms the assumption that autoclaving was unable to break down the chitin.

The insignificant differences in the feed efficiency values irrespective of the dietary treatments at varying levels of squilla meal suggest that inclusion of squilla meal has not adversely affected this vital economic parameter (Table 24).

The poor feed efficiency on autoclaved diets clearly established that autoclaving is not beneficial in improving the nutritive value of squilla meal. The significant lower FE values on raw squilla meal were expected because of the lower growth coupled with higher feed intakes.

The improvement in feed efficiency with enzyme supplementation confirmed that it is the most appropriate way to improve the utility of the squilla meal. Minor insignificant differences among different levels of enzyme supplementation point towards the fact that increasing enzyme level beyond 0.5 g/kg dietary level will not have any

further beneficial effect. Moreover considering economic aspects as well, it appears most appropriate to suggest 0.5 g/kg as the optimum enzyme supplementation level when complete replacement of squilla meal in place of fish meal was done. Improved feed efficiency by enzyme supplementation was also reported by Ranade *et al.* (1992) and Padmanabhan (1992).

5.2.1.2.3 Livability: Livability on different diets was comparable (Table 25), indicating the options of any deleterious or toxic factors in the test material.

5.2.2 Utilization of squilla meal for egg production (Experiment 3 and 4)

The influence of inclusion of squilla meal at 44.5, 88.9 and 133.4 g/kg at the expense of fish protein in layer diets (Experiment 3) and the inclusion of raw and autoclaved squilla meal (88.9 and 133.4 g/kg) and supplementation of enzyme premix (0.5, 1.0 and 2.0 g/kg diet) to the 88.9 and 133.4 g/kg of raw squilla meal diets (Experiment 4) are discussed below:

5.2.2.1 Experiment 3: Utilization of raw squilla meal in layers.

5.2.2.1.1 Hen-day egg production: The lowered hen-day egg production on highest level (133.4 g/kg) of squilla meal diet, may be due to lower levels of critical amino acid like

lysine and methionine (Table 43) and or higher level of chitin in the diet. With increase in the level of squilla meal, lysine content at 88.9 and 133.4 g/kg and methionine content at 133.4 g/kg diet became critical. This might have reflected in significantly ($P<0.01$) lowered production on 133.4 g squilla meal per kg diet. On this diet, the chitin content also was 18.5 g/kg. Ramachandra Nair *et al.* (1993) observed lowered egg production on diet containing as low as 5.0 g chitin per kg diet. Hirano *et al.* (1990) feeding chitosan in diet concluded that the safe dosage of chitosan was <1.4 g/kg body weight per day for hens and broilers. Assuming a body weight of 1.5 kg and feed intake of 110 g/h/d, the toxic dietary concentration of chitosan would be 19 g/kg feed. In this study, the diet containing the highest level of squilla meal had 18.5 g chitin per kg diet.

5.2.2.1.2 **Feed consumption and feed efficiency:** Inclusion of squilla meal in the diet did not influence feed intake. The numerically higher feed intake values observed on two higher levels (88.9 and 133.4 g/kg). The results obtained in this study are in accordance with the findings of Hirano *et al.* (1990). The feed consumption values of laying periods differed significantly ($P<0.05$). This may be due to the advance of age resulting in increased body weights. Such an increase in the feed intake with the advancement of age of bird is well documented in the literature (Mack O North, 1990).

Table 43: Essential amino acid composition (as such basis) of layer diets containing varying levels of squilla meal (Experiment 3 & 4).

Essential amino acid (%)	Fish based control diet	Fish protein replaced by squilla meal at (%)		
		33.3	66.6	100.0
Arginine	1.15	1.11	1.07	1.03
Glycine plus serine	1.60	1.58	1.56	1.54
Histidine	0.34	0.34	0.34	0.33
Isoleucine	0.57	0.56	0.55	0.54
Lysine	1.27	1.25	1.23	1.21
Leucine	0.66	0.62	0.57	0.53
Methionine	0.28	0.27	0.26	0.26
Phenylalanine	0.59	0.58	0.57	0.56
Tyrosine	0.51	0.51	0.51	0.51
Threonine	0.56	0.54	0.52	0.50
Tryptophan	0.16	0.16	0.16	0.15
Valine	0.74	0.73	0.72	0.71

The decreased feed efficiency on 88.9 and 133.4 g/kg squilla meal diets was due to increased feed intake and decreased egg production. The decreased feed efficiency might have also due to incomplete digestion of chitin. There is paucity of literature regarding the influence of squilla meal on feed efficiency in layers. However, the data of Ramchandra Nair *et al.* (1993) indicated a significant drop in egg production in chitin diet (55%) in comparison to control (70%). It is quite natural that the feed efficiency must have also lowered significantly. However, with other marine products like crab meal in layers no significant differences were observed in feed efficiency and egg external and internal quality parameters when fish based diets were compared. These variations in observations suggest role of non nutrients like chitin in the efficiency of utilization of feed.

5.2.2.1.3 **Egg weight:** Linolic acid remained more or less same in all the diets. Marginally lowered level of methionine might have played a part in significantly reduced egg weight on the diet containing higher level of squilla meal than on other diets.

5.2.2.1.4 Egg quality measures: The albumen index and Haugh Unit Score values due to treatments were higher in comparision to control (Table 30 and 31). Similarly, yolk index values were also better ($P<0.05$) than on control

except that on low levels (Table 32) but egg shell thickness was however not affected (Table 33). Generally, the quality parameters followed a reverse trend in relation to egg production. Such an inverse correlation of egg quality parameters with egg production and age of the bird is well established in the literature (Nesheim *et al.*, 1979).

5.2.2.2 Experiment 4: Utilization of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme in layers.

5.2.2.2.1 Hen-day egg production: The raw and autoclaved squilla meal at 88.9 g/kg in layer diets did not result in any adverse effects on the performance. However, higher level (133.4 g/kg) significantly ($P<0.05$) decreased egg production (Table 34). These results are in accordance to the earlier study of layer experiment. The decreased egg production on squilla meal diet at 133.4 g/kg level might be due to lower level of lysine and methionine and or higher content of chitin as discussed in detail in section 5.2.2.1.1.

The improvement in egg production on addition of bacterial proteases enzyme at higher level (133.4 g/kg) of squilla meal in diet, might be due to improved utilization of protein because of proteolytic enzymes. The enhanced performance of birds on enzyme supplementation (0.5 g/kg diet) group is obvious. However, no further response of

enzyme at higher levels in the diet did indicate, that the optimum level of inclusion was just 0.5 g/kg diet.

5.2.2.2.2 Feed consumption and feed efficiency: Feed consumption was not influenced significantly ($P>0.05$) on different dietary treatments except slightly higher values observed on higher levels of squilla meal diets. A similar increased feed consumption was also observed in previous layer experiment in these studies with higher inclusion levels of squilla meal in layer diets.

Feed efficiency values (Table 36) on higher level (133.4 g/kg) of squilla meal decreased significantly ($P<0.05$). Autoclaved squilla meal diets also had significantly lowered feed efficiency. On enzyme supplementation at 0.5 g/kg level to squilla meal diets restored the efficiency of feed utility values. The highest enzyme supplementation (2 g/kg) to squilla diet could not further improve feed efficiency values. Results obtained in this study are suggesting that a level as low as 0.5 g/kg enzyme is sufficient to improve feed efficiency in diets with higher level (133.4 g/kg) of squilla meal.

5.2.2.2.3 Egg weight: Egg weight was significantly ($P<0.05$) lowered on higher level (133.4 g per kg) of squilla meal and also on autoclaved squilla meal diet (Table 37) might have due to marginally lowered levels of methionine as discussed in 5.2.2.1.3. Egg weight improved on supplementation of

enzyme premix at 0.5 g per kg level to higher level of squilla meal diet and these values were comparable with control. This is an indication that enzyme premix supplemented diet improved protein digestion and availability of methionine + cystine which might have contributed to the improved egg weights.

5.2.2.2.4 Egg quality measures: The lowered Haugh unit scores on higher levels (133.4 g per kg) of squilla meal and also on the both levels of autoclaved squilla meal. The enzyme premix supplementation have improved the Haugh unit scores (Table 38). The results obtained on albumen index values showed no specific trend on different dietary treatments. Yolk index values were not influenced by dietary levels of squilla meal. Egg shell thickness values were lowered on treated groups, however enzyme supplementation improved shell thickness. The treatment and period interaction effect was not significant, indicating that no specific level of test ingredient performed well during all the experimental periods.

SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSIONS

Investigations were aimed to study the nutirtive value and utilization of squilla meal *vis-a-vis* fish protein for poultry. Squilla meal was evaluated for its nutritive value by *in vitro* and *in vivo* studies. The utilization was studied for growth in broilers and for production parameters in White Leghorn layers.

The squilla meal obtained locally contained 339.7 g per kg crude protein, which was comparatively lower than fish meal (546.0 g/kg). It was rich in total ash and trace minerals like manganese, zinc and iron than fish meal. Squilla meal had higher crude fibre Vis a vis chitin content (139.2 g/kg) than fish meal (32.0 g/kg). The metabolizability of energy in squilla meal was 7.13 MJ/kg as against 8.08 MJ/kg in fish. Its amino acid composition was generally lower in comparison to fish. The GPV of squilla meal was 68 per cent and that of fish was 72 per cent, while the protein digestibility was 66 per cent being less than that of fish, 74 per cent.

The squilla meal was incorporated upto 166.8 g/kg in the broiler diets replacing 25, 50, 75 and 100 per cent of fish protein. The body weight gains on squilla meal at various levels (41.7, 83.4 and 125.1 g/kg) were comparable

with the control diet (Experiment 1), except, at 166.8 g/kg (100 per cent), where in numerically lowered body weight gains were recorded. The feed intake was increased at lower levels of fish replacement and slightly decreased at higher levels of replacements which were still higher than control. Feed efficiency values were numerically higher with the increasing inclusion levels of squilla meal. The feed cost per kg live weight gain with all levels of inclusion of squilla meal was comparable. The weights of visceral organs like Liver, spleen, kidney and pancreas on test diets were comparable with fish based diet. Significant linear effects were observed in lenghts of duodenum and caecum with the increasing levels of squilla meal in diet.

The raw, autoclaved and enzyme supplemented squilla meal were incorporated at 83.4, 125.1 and 166.8 g/kg in broiler diets replacing 50, 75, and 100 per cent of fish protein. The raw squilla meal when replaced fish protein caused a significantly lowered weight gain than control (Experiment - 2). The feed consumption was numerically higher, while feed efficiency was significantly poorer than control.

Autoclaving of squilla meal failed to improve the performance in terms of weight gains and feed efficiency. Infact there was a drop in weight gains which indicated a significant (P=0.0449) linear negative trend.

Addition of bacterial proteases enzyme premix at 0.5 and 1 g/kg level to the squilla meal diets resulted in numerically higher body weight gains than control. While, at 2 g/kg level the weight gains were lower than above two levels tested but comparable with control. The feed consumption decreased on supplementation of enzyme premix compared to raw squilla meal diets but the figures, were still higher than control. The feed efficiency values showed a significant improvement with an enzyme supplementation.

Squilla meal was incorporated at graded levels 44.5, 88.9 and 133.4 g/kg, replacing 33.3, 66.6 and 100 per cent of fish protein in WL layer diets. The hen-day egg production upto 88.9 g/kg level was comparable to control, however at 133.4 g/kg replacement level it decreased significantly. The feed consumption was however unaffected, while the feed efficiency value at lower level was comparable but significantly poorer to control at 88.9 and 133.4 g/kg levels. Egg quality parameters were significantly influenced, without any specific trend.

The efficacy of raw and autoclaved squilla meal and raw squilla meal supplemented with enzyme was tested in WL layer diets at 88.9 and 133.4 g/kg levels. The hen-day egg production on raw and autoclaved squilla meal at 88.9 g/kg level was comparable with control. However, at 133.4 g/kg level significantly lowered egg production was recorded. The feed intake values were comparable. The feed efficiency

values on autoclaved squilla meal at 88.9 and 133.4 g/kg level and raw squilla meal at 133.4 g/kg level were significantly poorer than control.

Addition of bacterial proteases enzyme premix at 0.5 g/kg level to the raw squilla meal diet at 133.4 g/kg improved the performance in terms of hen day egg production, feed consumption and feed efficiency in comparision to unsupplemented raw squilla meal diet, but comparable to control. Further, enhancing the enzyme levels to 1·0 and 2·0 g/kg of diet did not cause any further improvement in the performance.

Conclusions

The following conclusions were drawn from the results obtained in these studies:

1. The protein content of squilla meal (339.7 g per kg) was lower than fish meal (546.0 g/kg) and protein quality in terms of GPV and protein digestibility for squilla meal was lower as compared to fish.

2. Squilla meal contained higher total ash (383.1 g per kg) than fish (314.5 g per kg) as such the test material is a good source for trace minerals than fish meal.

3. There was only marginal difference in ME content of squilla meal (7.13 MJ/kg) and fish meal (8.08 MJ/kg). The crude fibre content of squilla meal was 139.2 g per kg, which was higher than fish meal (32.0 g per kg).

4. Considering the growth pattern it appears that squilla meal can replace 3/4th fish nitrogen without adversely affecting growth, however, at total replacement (100 per cent level) weight gains were depressed.

5. Bacterial proteases enzyme premix supplementation (0.5 g per kg diet) to broiler diets could restore weight gains, even when squilla meal was used as a sole animal protein source. It has also improved efficiency of feed utilization in comparision to unsupplemented squilla meal diets and was comparable to control.

6. Inclusion of squilla meal at 44.5 and 88.9 g/kg replacing fish protein at levels of 33.3 and 66.6 per cent in layer diets resulted in comparable egg production with that of fish based control diet. However, inclusion at 133.4 g per kg replacing fish protein at 100 per cent level significantly ($P<0.01$) lowered egg production than control.

7. Performance of layers in terms of egg production and feed efficiency improved by supplementation of enzyme premix at 0.5 g per kg for higher squilla meal diet. Further, enhancing the inclusion levels of enzymes did not give any favourable response.

8. The incorporation of squilla meal, has generally resulted in higher feed intake than control, indicating that addition of squilla meal has not affected the palatability of the diets.

9. Autoclaving of squilla meal was not only ineffective in improving growth in broilers and egg production in layers, but infact caused significantly lowered performance.

10. In the wake of the above observations, it is concluded that squilla meal can be safely incorporated upto 75 per cent of fish protein in broiler diets and 66.6 per cent of layer diets. Enzyme supplementation (0.5 per kg diet) is required for complete replacement of fish protein for growth in broilers as well as egg production in layers.

LITERATURE CITED

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Abdelsamic R E, Ranaweera K N P and Nano W G 1983 The influence of fibre content on physical texture of the diet on the performance of broilers in the tropics. British Poultry Science 24: 283-290.

Anwar A 1960 A new method for calculating the gross protein value of proteins. Poultry Science 39: 1406-1408.

AOAC 1990 Official Methods of Analysis. Association of Official Analytical Chemists, Virginia, USA.

Anon 1983 Mineral analysis. pp. 136-137. *in* A manual of laboratory techniques (ed. N Raghuramulu, K Madhavan Nair and S Kalyana Sundaram), National Institute of Nutrition, Hyderabad, India.

Arai K, Kinumaki T and Fujita T 1968 On the toxicity of chitosan. Bulletin of Tokai Regional Fisheries Research Laboratories (Japan) 56: 89-95.

Austin P R, Brine C J, Castle J E and Zikakis J P 1981 Chitin : New facets of Research. Science 212: 749-753.

FAO/WHO 1973 Energy and protein requirements. Food and Agricultural Organisation of the United Nations, Rome. pp.63.

Gaering H K and Van Soest P J (Editors) 1970 Forage fibre analysis (Appartus reagents, Procedures and some applications). USDA Agriculture Hand Book No.379, Washington, DC, pp.5-9.

Govindan T K 1984 Squilla an untapped resource of India possessing vast economic potential. Sea food export 16: 15-18.

Heiman V, Carver J S and Cook J W 1939 A method for determining the gross values of protein concentrates. Poultry Science, 18: 464-474.

Hill F W and Anderson D L 1958 Comparison of metabolizable energy and productive energy determination with growing chicks. Journal of Nutrition 64: 587-603.

Hirano S, Hayashi M, Murae K, Tsuchida H and Nishida T 1988 Chitosan and derivatives as activators of plant cells in tissues and seeds. Polym Science Technology 38: 45-59.

Hirano S, Itakura C, Seino H, Akiyama Y, Nonaka I, Kanbara N and Kawakami T 1990 Chitosan as an ingredient for domestic animal feeds. Journal of Agricultural Food Chemistry, 38: 1214-1247.

Horn M J, Jones D B and Blum A E 1946 Determination of methionine. Journal of Biological Chemistry 16: 321-323.

Ilian M A, Bond C A, Salman A J and Al-Hoot S 1985 Evaluation of shrimp by catch-meal as broiler feed stuff. Nutritional report of International, 31: 487-492.

Jarvuin R, Braham J E, Gonzalez J M and Bressani R 1972 Nutritive value of by-products of shrimps for poultry. Turrialba 22: 160-167.

Jordon E L and Verma P S 1976 Invertabrate zoology. Sixth edition, Chand and Company Ltd., Ramnagar, New Delhi.

Kadam A S, Ranade A S, Rajmare B V, Dauge S H and Patil S S 1991 Effect of enzyme feed supplement on the performance of broiler. Poultry Advisor XXIV(ii): 21-24.

Kobayashi S and Itoh H 1991 Effect of dietary chitin and chitosan on growth and abdominal fat deposition in chicks. Japanese Poultry Science 28: 88-94.

Kondra P A, Sell J L and Guenter M 1974 Response of meat and egg type chicken to high fibre diet. Canadian Journal of Animal Science 54: 651-658.

Kratzer F H, Rajaguru R W A S B and Vohra P 1967 The effect of polysaccharides on energy utilization, nitrogen retention and fat absorption in chickens, Poultry Science 46: 1489-1493.

Lekshmy Nair A, Madhavan P and Prabhu P V 1991 Nutritional studies on the evaluation of the safety of squilla protein. Fishery Technology, 28: 142-145.

Lodhi G N, Renner R and Clandinin D R 1970 Factors affecting the metabolizable energy value of rapeseed meal nitrogen absorbability. Poultry Science 49: 991-999.

Lubitz J A, Fellers C R and Parkhurst R T 1943 Crab meal in poultry rations 1. Nutritive properties. Poultry Science 22: 307-313.

Mack O North and Bell D D 1990 Commercial chicken production manual, 4th Edition, An AVI Book of VanNostrand Reinhold, New York, USA.

Madhavan P and Ramachandran Nair K G 1975 Chitosan from squilla. Fishery Technology 12: 81-82.

Mathew P T, Lekshmy Nair A and Prabhu P V 1982 Squilla protein: Chemical composition and nutritive value. Fishery Technology, 19: 97-100.

McKittrick D C 1947 Selection of chicks for growth experiment and the evaluation of the growth. Growth 11: 989-999.

Menachery M, Ananthasubramanian C R and Nair A M C 1978 Feeding value of srimp shell powder (Prawn waste) for broiler chicks. Indian Journal of Nutrition and Dietitics 15: 381-388.

Nageswara Rao G 1983 Statistics for Agricultural Sciences. Mohan Primlani for Oxford and IBH publishing company, New Delhi.

Nandeesha M C, Basavaraja N, Keshavanath P, Varghara T J, Shetty H P C and Srikanth G K 1989 Influence of soyabean and squilla meal diets enriched with saradine oil on the growth and organoleptic quality of common carp (Cyprinus carpio). Biological Waste 30: 61-69.

Narahari D, Talukedar J K, Reddy P S, Sundaravasa V and Sundaresan K 1991 Performance promoting ability of chitin in chicken. Indian Journal of Animal Sciences, 16: 1016-1017.

Nesheim M, Austic R E and Card L E 1979 Poultry production. Lea and Febiger, Philadelphia, P.A.

Padmanabhan M K 1992 Use of enzymes in Poultry. Indian Poultry Review 23(12): 47.

Parkhurst R T, Gutowska M S and Fellers C R 1944 Crab meal in poultry rations. 3. Laying and breeding rations. Poultry Science 23: 118-125.

Pons W A, Cucocullu A F, Lee L S, Robertson J A, Franz A O and Goldblatt L A 1966 Determination of aflatoxins in agricultural products use of aqueotts acetone for extraction. Journal of the Association of Official Agricultural Chemists, 49: 554-562.

Potter L M and Shelton J R 1973 Evaluation of crab meal as a dietary ingredient for young turkeys. British Poultry Science, 14: 257-261.

Rajashekher A R, Rao P V, Reddy V R and Reddy V R 1993 Utilization of Ambadi meal (*Hibiscus cannabinus*) by growing chickens. British Poultry Science 34: 339-349.

Rajashekhar Reddy A 1989 Studies on the nutritive value and utilization of Ambadi (*Hibiscus cannabinus*) cake in poultry feeds. Ph.D. thesis, submitted to Andhra Pradesh Agricultural University, Hyderabad.

Ramachandran Nair K G, Mathew P T, Madhavan P and Prabhu P V 1987 Chitin as a feed additive for broiler chicken. Indian Journal of Poultry Science 22: 40-44.

Ramachandran Nair K G, Mathew P T, Madhavan P and Prabhu P V 1993 Effect of feeding chitin on poultry. Fishery Technology, 30: 24-27.

Ranade A S, Kulkarni A A, Girdhar S R, Rajmane B V, Deshmukh and Kadam A S 1992 Effect of enzyme feed supplement on commercial broilers. 14th All India Poultry Science conference Avain Nutrition and Feed Technology: 24.

Rand N T, Collins V K, Varner D S and Mosses J D 1960 Biological evaluation of the factors affecting the protein quality of fish meals. Poultry Science 39: 45-52.

Ranjhan S K 1993 Animal nutrition in the tropics, third edition. Vikas publishing house, Jangpura, New Delhi.

Rao S R, Cartes F L and Frampton V L 1963 Determination of available lysine in oil seed meal proteins. Analytical Chemistry 35: 1927-1930.

Ravinder Reddy S 1993 Studies on the nutritive value and utilization of differently processed sunflower seed meal in poultry feeds. Ph.D. thesis, submitted to Andhra Pradesh Agricultural University, Hyderabad.

Rexen B 1981 Use of enzyme for improvement of feeds. Animal Feed Science and Technology 6: 105-114.

Richards A S 1953 Chemical and physical properties of cuticle : chitin and its derivaties. Page 23 in Insect physiology. K.D. Roeder Ed. John Willey and Sons, Inc., New York, NY.

Savary L T and Gentle M J 1976 Changes in food intake and gut size in Japanese quails in response to manipulation of dietary fibre content. British Poultry Science 17: 561-570.

Sibbald I R and Slinger S J 1963a A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. Poultry Science 42: 313-325.

Snedecor G W and Cochran W G 1967 Statistical methods, 6th edition, IBH Publishing Company, Calcutta.

Spies Jr and Chamber R C 1948 Determination of tryptophan. Analytical chemistry 20: 30-32.

Spreen K A, Zikakis J P and Austin P R 1984 Proceedings Jt. U.S. Jpn., Seminar Advances chitin, Chitosan related enzymes, 57th Ed. Zikakis, J.P., Academic, Orlando Fld.

Stelmock R L, Husby F M and Brundage A L 1985 Application of van soest acid detergent fibre method for analysis of shellfish chitin. Journal of Dairy Science, 68: 1502-1506.

Sukumaran K K 1987 Study on the fishery and biology of the Mantis shrimp *Oratosquilla nepa* (Latreille) of South Kanara Coast during 1979-83. Indian Journal of Fisheries 34: 292.

Sukumaran K K 1988 Stomatopod resources of the south kanara coast during 1983-86. Marine fisheries information service 83: 19-21.

Tolokonnikov Yu, Oralov L V, Nikilburskil N I, Stratichuk A N and Vedeneeva V A 1984 Shrimp meal in diets for meat chicken. cited in Nutrition Abstracts and Reviews, 1986: 56-3260.

Zikakis J P, Saylor W W and Austin P R 1982 Utilization of chitinous products and whey in animal nutrition. Proceedings of the second international conference on chitin and chitosan, Sappoaro, Japan, 223.