11 REVIEW OF LITERATURE

The Indian silk industry is now gathering momentum to become a prominent player in global market, china is the world’s largest producer of silk contributing around 85 per cent of the global produce, India stands second supplying around 13 per cent. In India a significant progress has been made in the production of raw silk, where production has reached to 18,475 tonnes during the end of 2006-07, as against 15,742 tonnes in 2003-04. India also has the distinction of producing all the known varieties of silk viz mulberry, Eri, Muga, and Tasar. Traditional silk producing states are mainly Karnataka, Andhra Pradesh, Tamilnadu, west Bengal and Jammu and Kashmir, which produce more than 80 per cent of the raw silk production, recording a growth weight rate of 6.41 per cent and out of this Karnataka alone produced 42 per cent which is about 7,883 tonnes, becoming the largest silk producing state. The exports of silk and silk products are shown in Table 1.

2.1 Quantification of silkworm pupae.

Ichhponani and Malik, (1971) reported that in India the annual production of 20,000 metric tons of silkworm pupae meal which makes it useful, as source of proteins. Shivaprakash, (1988) estimated that, more than 10 million kilograms of pupae are available every year in Karnataka alone. A small quantity of this volume is now being used for oil extraction Roy Choudhury and Joshi ,(1995) reported that silkworm pupae are by-products of reeling industry and It is estimated that annually 1.5 lakh metric tones of pupae are produced which is considered as waste material. India being one of the leading silk producers in the world which produced 14,048 tones of mulberry raw silk, with about 10,000 tones of silk waste during 1997-98. India produces, 1,16,672 tones of green mulberry cocoons out of which 20 per cent is dry pupae weight.

Hence 23,334 tones of dry pupae is available per year silkworm pupae constitutes 60 per cent of dry cocoon weight, hence 23334 tones of dry spent silkworm pupae is available in the country. Since pupae are reported to have 50 per cent protein and 30
per cent fat, around 12,000 tones of protein and 700 tones of fat are likely to be available from the pupae, as by product of silk industry in India. According to Bongale, (2001) Karnataka state alone contributes major portion of cocoon production to the country’s production, which is about 60 per cent, 80,000 tons of cocoon are produced from 1, 20,000 hectares of mulberry cultivation. Out of 80,000 tones of cocoon production in Karnataka, 26400 tons of dry cocoon (33 per cent of cocoon weight), 15840 tons dry pupae (60 per cent of dry cocoon weight), 7920 tons of protein (50 per cent of protein in dry pupae) 4752 tons of fat (30 per cent fat in dry pupae) can be obtained. Approximate calculations for the yield of amino acid mixture are presented in Table-2.

2.2 Uses of Silkworm pupae meal

The mulberry silkworm pupa is one of the major by-products of silk industry. This is considered as waste in silk reeling unit. Disposal of silkworm pupae meal was a big problem in the silk factories. The silk producers after reeling out silk used to throw the dead pupae at the outskirts of the city, creating nuisance and health hazards. A great deal of work has been done on silk waste but no appreciable value of work has been done on pupae-waste until 1959, even though the mulberry silkworm pupae consist of numerous biological constituents which are of great value as feed / food for animals including human beings, medicine and manure for crops. Later Naidu (1959) stated that powdered silkworm pupae meal would make a good protein supplement for poultry mashes. Later several workers carried out investigation on utilization of silkworm pupae meal as animal protein source for varied purposes, Since Pupae are a good source of proteins, amino acids, fat, carbohydrates and vitamins, besides it is rich in oil. These constituents of pupae are being used in diverse sectors for various purposes. As such, pupae are one of the best sources for animal feed (fish and poultry) medicinal and manure purposes.

A) Feed value for animals.

Naidu (1959) stated that the powdered silkworm pupae would make a good protein supplement for poultry. During 1965, scientists at central food and technological research institute, Mysore were the first to show the importance of silkworm pupae meal as potential source of animal protein in livestock feeds. The
refined protein pupa was superior to that of fishmeal and about equal to that of beef as cited by Nanavathy (1965). Nanda (1967) carried out a pilot study, on this and indicated that the processed silkworm pupae was an excellent source of protein, Panda (1967) carried out an investigation and showed that, it was possible to manufacture silkworm pupae meal on large scale. Nataraj and Basavanna (1969) reported that the protein concentration of pupae can be used to supplement the poultry feed and in cattle feed and reported that this meal might be an important substitute for fish meal.

Panda (1970) reported that the nutritive value of silkworm pupae meal was found to be 134 per cent superior over casein and its pepsin digestibility was 89.5 per cent when used as feed for poultry. Ichhponani and Malik, (1971) reported a beneficial effect in chicks by replacing five per cent of fishmeal and 10 per cent of GNC together with silkworm pupae meal he included the silkworm pupae in the diets of chicks and found that Metabolizable energy content of the diet was not affected due to inclusion of pupae in the meal. Saikia et al., (1971) studied the egg production capacity of several cheap mashes using by products of agro-industrial origin and concluded that mashes containing deoiled silkworm pupae meal, meat meal, molasses, distillery waste and soybean were found to be much superior to the standard rations containing 26 per cent maize, eight per cent ground nut cake and five per cent fishmeal. The mash containing deoiled silkworm pupae meal gave the best performance among the experimental rations used. Lodhi and Ichhponani (1974) reported the absorbability of protein in deoiled silkworm pupae meal as 57 per cent and 77 per cent when 50 per cent of total nitrogen was replaced by ground nut cake and fish meal in poultry diet. Panda and Rao, (1975), replaced 10 per cent of maize and entire portion of fishmeal with deoiled silkworm pupae meal and found satisfactory growth rate in young stock and egg production in layers. Joshi et al., (1980) studied the effect of substitution of various levels of deoiled silkworm pupae meal (both quantitative and isonitrogneous) replacing fishmeal on performance of layers, they also reported substitution of fishmeal by silkworm pupae improved feed efficiency over the control.
Virk et al., (1980) conducted experiments to assess the nutritive value of untreated, acid and water treated deoiled silkworms pupae meal as a substitute for fish meal in broiler diets. Based on their results on body weight gain, feed and protein conversion ratio they concluded that untreated silkworm pupae can replace 50 and 75 per cent fish meal protein in broiler starter and finisher diets respectively. Fagoone (1983) suggested for substitution of silkworm pupae in chick diets in place of fish meal, meat and bone meal. Fagoone (1984) studied the effect of inclusion of dried and ground spent silkworm pupae on broiler performance. Chicken were given a diet of maize 51 per cent, wheat bran 15 per cent, groundnut oil meal 14 per cent, fishmeal 10 per cent, pollard four per cent, meat and bone meal four per cent minerals and vitamins two per cent. The test groups were given diets containing dried and ground spent silkworm pupae with half or all of fishmeal replaced by silkworm pupae meal. He concluded that total replacement of fishmeal had adverse affect on growth rate performance.

Tas (1987) also suggested for substitution of silkworm pupae meal in chick diet in place of fish meal, meat and bone meal. Mathur et al., (1988) reported that silkworm pupae refined protein is superior to that of fishmeal and beef protein in the diets. According to Mishra and Das (1992), the refined protein of silkworm pupae was superior protein for rat and fish diets over fishmeal. They also concluded that due to presence of Vitamin B$_1$ and B$_2$, nicotinic acid and folic acid and as such formed excellent sources of protein and vitamins for livestock.

Singh et al., (1992) reported that dead Tasar silkworm pupae and moths can be used as fish feed. Fish fed with Tasar pupa showed significant increase in body weight against the control fed with fishmeal. There are however some reports on the use of pupae-waste in poultry feed, in manure and in the preparation of fish-food Datta and Majumder (1993). Datta et al., (1993) reported that about 25per cent oil could be extracted from pupae-waste. Swamy (1994) reported that Silkworm pupae that contain fat and protein was superior to plant proteins when fed to carp fish. In Japan, silkworm pupal cakes are being prepared and used as feed for cattle, pig and fowls. Rabbits fed with the silkworm pupae resulted in increased fat deposition and fur growth rate significantly (Aruga 1994). Both oiled and deoiled pupae forms good
source of animal protein and hence used in feed mixture, there is controversy among the workers as to the suitability of silkworm pupae meal as a total or partial substitution for soybean in poultry rations.

**B) Human consumption**

Silkworms provide not only silk fiber but also food to human beings. Bristowe (1932) has mentioned in his comments that the Chinese eat silkworm’s pupae. In some parts of India and China, the silkworm pupae are regarded as delicious food and are extensively eaten when the silk has been reeled off. The pupae are either cooked in very hot water or roasted. It is a delicacy to tribal’s in some parts of Northeastern States of India. The fresh larvae, prepupae and pupae of non-mulberry silkworms such as muga, tasar and eri are preferred as food by Garo, Mikir and Khasi tribes of India. They are in high demand in local markets of Northeastern State. The silkworm pupae protein is of great value and can be better used in baking industry for preparation of protein rich biscuits. Silkworm pupae enriched protein biscuits contained six per cent nitrogen and 37 per cent protein (Anon., 1967).

About 40 kg of pupal oil and 160 kg of white protein powder can be obtained from 200 kg of tasar pupae. Silkworm pupae are used as food by human beings in northeastern region of India Mathur et al., (1988). According to Majhi et al., (1991), the live silkworm pupae are eaten by tribal population of northeastern region and Chotanagpur of Bihar. They also stated that consumption of pupa by tribes followed declining trend and opined that oil free pupa powder could be used for preparing protein rich biscuits. While the tribes of Garo, Mikir and Khasi of India prefer fresh larva, prepupa and pupa of Eri, Muga and Tasar as food (Roychoudhury and Joshi, 1995).

**C) Medicinal value.**

Chitosans is a well known derivative of chitin which is a deacetylated derivative of chitin, it is chemically known as poly (2 amino-2-deoxyglucose) the degree of deacetylation refers to the relative amount of acetyl groups removed from the molecule of chitin (Bergman et al., 1935). Chitin can be converted into various useful products like chitosans, chitin sulphate, chitin nitrate, chitin xanthate, sodium carboxymethyl. Chitin can be hydrolyzed by enzyme and anhydrous formic and
mineral acids. Biologically properties of chitosans are Biocompatibility, Haemostatic, Bacteriostatic, Fungistatic, Spermicidal, Anticancer and Anticholesteremic. Chitin, chitosans and their derivatives have applications as wound dressings, controlled release of drugs and in contact lenses.

D) Manurial value.

Dried silkworm pupae contain about 8 per cent nitrogen. The crude protein extracted with 0.5 per cent sodium hydroxide contains 12.22 per cent nitrogen. Silkworm pupae used as manure to the mulberry in three different forms viz. raw pupae, raw pupae powder and deoiled pupae powder.

Bora and Sharma (1965) reported that calcium and phosphorous contents in silkworm pupae (Assam muga silkworm) as 0.26 and 0.80 per cent respectively. Application of pupae resulted in significantly greater root and shoot weight. Maximum root, shoot and leaf yield recorded in deoiled pupae powder with increased crude protein and reduced mineral content in mulberry leaf. (Anon 1967). Nagaraj and Basavanna (1969) reported that deoiled residue consists of 10 per cent of nitrogen. While protein extraction with 0.5 per cent sodium hydroxide solution was good resulting in recovery of 12 per cent nitrogen. Krishnaswamy et al., (1973) reported that silkworm pupae were very rich in protein and fat content and felt that it can be used as manure. Rajashekar and Obilami (1981) reported that eri silkworm pupae contained 11 per cent of nitrogen in deoiled pupal powder. Singh and Panda (1987) also reported that calcium and phosphorous content in pupae was 0.29 and 0.58 per cent, respectively. Mishra and Das (1992) is of the opinion that dead silkworm larva, pupae and moths can be effectively used in organic compost making them rich in biological constituents. According to Jain (1998) the major and minor mineral nutrients of oiled and deoiled silkworm pupae of manurial are shown in Table10a. In general, amino acids, the physiological important substances are prepared from high protein containing material, such as Soybean, wheat flour (gluten flour), blood, gelatin, casein and human hair that are generally costly materials.
2.3 Soybean meal

Soybean protein is invariably the standard against which other vegetable proteins are judged. Over the years, there has been a steady decline in guaranteed protein level, and there seems to be more variation than in the past. When combined with corn, methionine is often the only limiting amino acid. Soybean, like most vegetable protein concentrates, tends to be dusty and this can cause problems with some high protein mash diets. There is also some concern over the effect of high levels of soybean meal in the diet, on foot pad problems in certain classes of stock.

A relationship has been documented between high levels of soybean meal in the diets for broiler breeders and incidences of foot pad lesions in birds on litter. Limiting the level of soybean meal in diets for young turkeys is sometimes recommended again because of footpad lesions. The complex oligosaccharides component of soybean meal has been suggested and there is still some controversy regarding the ideal processing conditions of soybean about the residual trypsin inhibitor and urease content. Recent evidences suggests that a urease value of zero does not always indicate that soybean meal has been overcooked, and that lysine in soybean is quite stable even at relatively high cooking temperatures. A urease index of greater than a 0.5 rise in pH will likely to cause poor growth weight and reduced nutrient digestibility.

The availability of animal protein source especially fishmeal, both of quality and quantity at a reasonable price is a problem. Many a times fishmeal is adulterated with urea and salt. They may be contaminated with bacteria depending upon processing conditions and because of fishy odor its use is limited. Pupa which is also a high proteinaceous material which can be utilized as a low cost raw material for the synthesis of amino acids mixture on industrial scale. The comparative chemical composition of soybean meal and silkworm pupae meal is shown in Table-3. In Table 4 the proximate composition of Assam muga, Eri, Tasar and Squashed silkworm pupae meal are shown.
2.4 General Chemical composition of different types of silkworm pupae

2.4.1 Full fat silkworm pupae:

Dried silkworm pupae contained eight per cent nitrogen, which shows that its protein content is 50 per cent. The pupae contain 55 per cent crude protein, 25 per cent ether-extract and three per cent crude fiber (Panda 1968). According to Nataraj and Basavanna (1969) analysis of pupae revealed that it contains eight per cent ether-extract and three per cent crude fiber, 20-25 per cent of oil and five per cent mineral matter. The remaining 20-25 per cent is composed of carbohydrates and other organic matter. The silkworm pupae collected after reeling operation contained 70-75 per cent moisture and is usually subjected to sun drying. The dried pupa contains 10 per cent moisture, 55 per cent protein, 25-27 per cent fat and three per cent crude fiber as reported by Panda (1970). According to Bureau of Indian standard, (1971) the composition of full fat silkworm pupae is as follows:

<table>
<thead>
<tr>
<th>Proximate composition</th>
<th>Silkworm pupae meal (per cent max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>10</td>
</tr>
<tr>
<td>Crude protein</td>
<td>50</td>
</tr>
<tr>
<td>Crude fat</td>
<td>25</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>3</td>
</tr>
<tr>
<td>Total ash</td>
<td>5</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1</td>
</tr>
</tbody>
</table>

Fagoone (1983) reported that silkworm pupae, oven dried at 80°C contain 90.9 per cent dry matter, 47.9 per cent protein, 27.0 per cent fat, 3.4 per cent fiber and 5.6 per cent total ash. Majaonkar and Bjambure (1987) reported that silkworm pupae contain 48.12 per cent protein, 34.20 per cent ether extract, 1.84 per cent crude fiber, 11.40 per cent nitrogen free extract and 4.44 per cent total ash on dry matter basis. According to Jain (1988) proximate composition of fullfat silkworm pupae untreated and acid treated are shown in Table 5.

2.4.2 Deoiled silkworm pupae

The crude protein extracted with 0.5 per cent sodium hydroxide contained 12.22 per cent nitrogen, which showed that the protein content in the sample was 76 per cent (Anon., 1967). Panda (1970) published the proximate composition of deoiled
silkworm pupae with the values of the protein, lipids and crude fiber are 68.7, 2.5 and four per cent respectively. Chopra et al., (1971) reported that deoiled silkworm pupae contain 76 per cent crude protein.

Sujatha (1979) analyzed the deoiled silkworm pupae samples before and after autoclaving and reported that they contain 74.95 per cent and 71.21 per cent crude protein levels, respectively. Joshi et al.,(1980), also analyzed the deoiled silkworm pupae and reported that it contains 73.41 per cent crude protein. Lin et al., (1983) treated dried silkworm pupae with hydrogen peroxide and reported that the treated pupa was better in odour, taste and colour as compared to untreated ones. Further, they reported that treated meal contains 81.60 per cent protein, 6 per cent fat, 2.1 per cent ash and 23.2 ME of energy per kg on dry matter basis. Bose and Majumder, (1990) reported that deoiled pupae contained water (5.89 per cent), fat (0.47 per cent), protein (72.82 per cent), glycogen (6.92 per cent), chitin (5.55 per cent) and vitamin (5.48 per cent). According to Aruga (1994) the Chemical composition of deoiled silkworm pupae contained 5.49 per cent water, 72.82 per cent protein, 0.47 per cent fat, 6.92 per cent glycogen, 5.55 per cent chitin, 3.72 per cent ashes and 5.48 per cent others in pupae after extraction of oil. According to Bureau of Indian Standard, (1971) the composition of Deoiled silkworm pupae is as follows:

| Proximate composition Deoiled Silkworm pupae meal (per cent max) |
|------------------|------------------|
| Moisture         | 10               |
| Crude protein    | 65               |
| Crude fat        | 3                |
| Crude fiber      | 4                |
| Total ash        | 6.5              |
| Acid insoluble ash | 1.5             |

2.4.3 Protein and Amino acid composition of oiled and deoiled silkworm pupae.

After removal of oil, remaining part of the pupae contained water-5.89 per cent, fat-0.47 per cent, protein-72.82 per cent, glycogen-6.92 per cent, chitin-5.55 and vitamin -5.48 per cent. Refined proteins of pupae is superior to the protein of fishmeal and not inferior to that of beef. For the isolation of the amino acids, the pupae protein is hydrolyzed by boiling it with concentrated 6n HCL at 110°c for
16 hrs and bound and composition has been studied by pupae chromatography. The lowered food intake of animals on pupal diet may be due to the bad odor of the pupae meal or the presence of pupal hormone (ecdysone).

Panda (1970) reported the content of only six amino acids in silkworm pupae meal, the values being lysine 5.7, leucine 2.0, isoleucine 3.4, methionine 4.4, phenylalanine 2.7 and valine 3.8g per 100g protein.

Chopra et al., (1971) reported that deoiled silkworm pupal powder contains 5.36 per cent lysine. Where as Joshi et al., (1980) reported 5.36 per cent lysine and 2.39 per cent methionine on per cent dry matter basis. Fagoone (1983) observed variation in the concentration of some essential amino acids in pupae of various stages (0, 2, 4, 6 and 8 days old). He reported that lysine content ranges from 2.62 to 3.21 per cent, arginine from 1.56 to 1.98 per cent glycine 2.3 to 3.61 per cent, methionine from 0.96 to 1.46 per cent, isoleucine from 1.67 to 1.93 per cent and leucine from 28.4 to 32.5 per cent.

Lin et al., (1983), treated dried silkworm pupae meal with hydrogen peroxide and reported that the treated meal was better in odour, taste and colour as compared to untreated ones. The amino acid value of silkworm pupae (either water or hydrogen peroxide extracted) is lower than casein except for tryptophan and sulphur containing amino acids. Hence water treated silkworm pupae meal is better suited for eventual use as food component. Chemical composition of pupae as varied with extraction methods is presented in Table-6. The total amino acid obtainable from pupal powder is 10 per cent of the pupal powder Datta et al., (1993). Oiled and Deoiled silkworm pupae analyzed for amino acid composition by Jain. 1988, Bose and Majumder. (1990) and Datta et al., (1993). The details of Composition are presented in Table-7 and Paper chromatographic analytical data of the amino acids mixture of silkworm pupae meal are given in Table-8. According to Udayshekara Rao (1994), the amino acids lysine, threonine, methionine and tyrosine in silkworm pupae meal are higher than in whole egg protein. Spent silkworm pupae meal contained 48.7g per cent crude protein, whereas defatted silkworm pupae meal contained 75.2 per cent crude protein. The essential amino acid content of pupal protein was similar to that of whole egg protein with exception of tryptophan 0.9g
per 16 g of nitrogen. Tryptophan is the limiting amino acid of pupal protein, chemical score of protein was 60, as compared to 100 for whole protein. The protein quality of spent silk worm pupae meal was significantly lower than casein (milk protein) as judged by Protein efficiency ratio and Net protein utilization (Losclevich et al., 2004).

2.5 Fat:
Spent silk worm pupae contained 30 per cent fat, Fatty acid composition of ether extract is characterized by a high percentage of Poly unsaturated fatty acids (11 per cent C18:3). The fatty acid profile of silkworm pupae meal reveal 66.8 per cent of total Unsaturated fatty acid and 29.9 per cent of Polyunsaturated fatty acids, with unusual higher content of linolenic acid (25.78 per cent) being exception among animal sources. The presence of high content of unsaturated fatty acids and linolenic acid leads to development of rancidity which is responsible for unpleasant odour (Bergan 1935). Bombyx mori pupae has iodine number of 110 and saponification number of 210 which is comparable to that of sunflower oil where as Antheracea assamensis pupae has got saponification, acid and ester value of 190.2, 131.2 and 59 respectively. Silkworm pupae meal fat has a lecithin content of 2.12 per cent and contains 20.7 per cent saturated fatty acid and 70.1 per cent unsaturated fatty acids. (Agri world- 2007)

2.5.1 Extraction of pupae oil from pupae powder:
During the biological development, pupae synthesize oil which can be extracted from powdered dried pupae by two techniques.

1) Solvent extraction method
2) By applying pressure

Oil content of pupae powder:
The process of oil extraction is simple and comparable to extraction of oil from many other products. The dried and cleaned pupae are first soaked in solvents like hexane, benzene, alcohol, chloroform, ether, petroleum ether etc in a soxhlet apparatus, followed by filtering and evaporating the solvent. Extraction of oil can be done with solvent under hot/cold conditions. The per cent of oil extracted by different solvents is presented in the Table-9. It shows that the hot extraction method
gives more per cent of oil than that obtained by cold extraction. Chloroform is the best solvent among the solvents used.

Silkworm pupa is rich in oil content. According to Anon (1967) 20-25 per cent of oil can be extracted from pupae. Krishnaswami et al., (1973) through solvent extract obtained 22-27 per cent pupal oil on dry weight basis. Jolly et al., (1974) reported that 20-25 per cent of oil can be obtained from Tasar silkworm pupae; Dried and Cooked Tasar silkworm pupae contained 25 per cent of oil (Jolly et al., 1981). Further, Mathur et al., (1988) extracted 27-30 per cent pupal oil on dry matter basis, whereas oil yield was 16 per cent on fresh matter basis.

As stated by Mathur et al., (1988) Italy is the second largest producer of pupal oil following Japan. The tasar silk production is very much restricted to northeastern states of India. The cocoons are reel able and pupa forms waste of silk reeling unit. Similar to Bombyx mori L pupae, tasar pupae are also rich in various biochemical constituents. Bose and Majumdar (1990) used ether for extraction of pupal oil in both hot and cold extraction methods. The oil recovery percentage was 25.1, 25.8, 26.3, 29.0, 26.3 and 26.7 in hot extraction while it was 24.7, 23.0, 25.7, 26.7, 24.3 and 26.5 per cent with cold extraction when petroleum ether, hexane, ether, chloroform, benzene and alcohol were used as solvents, respectively.

2.5.2 Properties of silkworm pupae oil and its use.

The freshly extracted silkworm pupae oil appears brownish tinge with fishy odour, while the refined oil has fluorescent yellow color and odorless. Anon (1967) prepared soap by using pupal oil as the saponification values are closely corresponded to the oils used for manufacture of soaps. The pupal oil had saponification number of 203, Iodine number of 45.02, unsaponifiable matter of 3.08 per cent, acid value of 11.53 g and RM value of 2.80. The saponification and iodine number of some oils (John-1967) are given in Table-10. According to Mathur et al., (1988) the pupal oil is dark brown in colour with fishy smell and that pupa oil can be mixed with linseed oil (25:75) for the manufacture of paints and varnishes. Besides, sterols separated from oil are used for preparation of hair tonic, high grade soaps, candles and in textile industry. While Bose and Majumdar (1990) reported that the pupal oil colour is transparent brownish tinge and
with fishy odour. The pupal oil is acidic in nature with a pH of 6.0. It does not emulsify with water but can be emulsified with sodium carbonate solution. The pupal oil is soluble in ether, petroleum ether (BP-40°C), Acetone, Isopropanol, Xylene, Carbon tetrachloride, Absolute alcohol, Glacial acetic acid, Ethyl acetate, N-Propanol and partially soluble in rectified spirit, formic acid and ciaxine.

2.5(2a) Oxidative stability of silkworm pupae:

Total tocopherol in total lipid is 125.2µ/g lipid; the oxidative stability of the total lipid from silkworm pupae was very high. It has been suggested that a synergistic effect between phospholipids and tocopherol in silkworm pupae meal plays an important role in protecting the lipids against oxidation. The silkworm pupa contained carotenoids such as lutein and neoxanthin. These carotenoids may act as antioxidants in total lipids (Eiichi Kotake-nara, et al., 2002).

2.5(2b) Purification of pupae oil

The oil can be refined by steam distillation or by filtering through activated charcoal or fuller’s earth. Removal of the odour from the oil is not the problem; the problem is related to the economics of the whole process. The oil extracted is brownish with fishy odour which can be cleared off by passing steam through the oil (Saratchandra, 1988). The refined oil may be utilized as an alternative to edible and Dalda as observed by Manjhi et al., (1991).

2.6 Mineral composition of silkworm pupae meal

According to feed technology article (Anon-2007) Mineral composition of silkworm pupae meal are calcium-0.63 per cent, phosphorus-1.25 per cent, sodium-0.03 per cent, and potassium-1.07 per cent. The major and minor mineral nutrients of oiled and deoiled silkworm pupae composition is given in Table-10.1.

2.7 Vitamins

Silkworm pupae meal is also very rich in vitamins, such as vitamin B1, B6, nicotinic acid, folic acid, and vitamin D. According to Feed technology article (Anon-2007) Silkworm pupae meal contains (mg/100g) Vit E-1000, Vitamin B1 - 15, Vitamin B2- 80 and Vitamin B12-0.5.

2.8 Pupa skin:
Pupae skin is one of the high potential commercial raw materials for various industries. It is available in the reeling and grainage industries as a waste. Although being a product of animal origin, remarkable fiber content was found due to the presence of chitin and other insoluble protein. Pupa skin is made up of Chitin, which is a polysaccharide and its structure is similar to that of cellulose. Pupae skin too is being thrown as a waste, causing pollution problem. Chitin always occurs in combination with other substances like proteins, calcium carbonate and a number of pigments. Some of these proteins are extractable with hot water and some are tightly bound with chitin.

2.8.1 Properties of chitin

Chitin is a white material resembling paper pulp. It is insoluble in water, dilute acids, dilute and concentrated alkalies and all organic solvents but it is soluble in anhydrous formic acid, hypochlorite solution and concentrated mineral acids (Dietrich Knorr, 1986). The degree of polymerization of chitin ranges from several hundred to one thousand n acetyl glucosamine residues. The reactive of chitin varies from sample, depending upon the preparation and the degree of crystallinity. Chitin can be converted into various useful products like chitosan, chitin sulphate, chitin nitrate, chitin xanthate, sodium carboxymethyl chitin it can be hydrolyzed by enzyme acids. Chitin is a polymer that can be found in anything from the shells of beetles to webs of spiders. It is present all around us, in plant and animal creatures. It is sometimes considered to be a spinoff of cellulose, because the two are very molecularly similar. Cellulose contains a hydroxyl group, and chitin contains amide. Chitin is a long unbranched polysaccharide. It is poly [n-acetyl- 2-deoxy-beta-d-glucopyranosel] and can be regarded as a derivative of cellulose, in which the hydroxyl group on the c.2 position has been replaced by the acetyl amino group – NHCOCH3.Chitin is unusual because it is a "natural polymer," or a combination of elements that exists naturally on earth. Usually, polymers are man-made. Crabs, beetles, worms and mushrooms contain large amount of chitin. Chitin is a very adaptive material for a creature. Insects and animals with chitin coats usually shed these coats, or molt, at least once a year. Chitin is a very firm material, and it help protect an insect against harm and pressure. Depending on its thickness, chitin can
be rigid or yielding. Often, insect coats contain thick, stiff layers of chitin. The areas around legs and face contain very thin, pliable layers.

2.8.2 Chitosan

Chitosan is a well known derivative of chitin and it is a deacetylated derivative of chitin. It has also been reported that chitin can be 100 per cent decatylated. acetone. Zikakis et al., (1982). It is chemically known as poly (2amino-2-deoxyglucose) the degree of deacetylation refers to the relative amount of acetyl groups removed from the molecule of chitin. Ramachandra Naik et al., (1993). It is not a single substance, but a group of partially decacetylated products of various degree of polymerization chitosan is insoluble in water, concentrated alkalies, alcohol and acetone unlike chitin, it is soluble in dilute acids it can be converted into chitin by n-acetylating it because of its solubility, it is available for commercial use.

2.8.3 Application of chitin and its derivatives

Chemical properties of chitosan are

1. Cationic polyamine
2. High molecular weight linear polyelectrolyte
3. Chelates certain transitional metals
4. Amiable to chemical modifications

The general uses of chitin and chitosans are oil refinery, waste water contaminant. Anticoagulant vascular grafts sutures, aggregation of cells, artificial kidney membrane. Feed supplement digestive aid promotion of growth of bifido bacteria (probiotic) cheese processing, breweries, flavour productions, food thickener and Paper making additive for surface strength. Removal of radioactive metals, heavy metals, colour and environment contaminants. Inhibition of plant growth and suppression of plant parasites. Chitin is present naturally to a level of 10per cent in certain marine protein feedstuff like squilla and prawn wastes. Hence they can be used on regular basis in fish meal preparation as a source of chitin in addition to its protein content. The ability of chitin in lowering the lipid content in blood, meat and egg can be explored to produce low fat meat and eggs at a cheaper cost.

2.9 Enzyme supplementation.
The enzyme–induced improvement in the nutritive value of poultry diets is well documented and it is generally conceded that the improvement in performance in relation to enzyme supplementation is due to the hydrolysis. Enzymes are proteins with highly complex three dimensional molecular structures, they are biological catalysts and act under very specific reaction conditions (Temperature, pH and Humidity) only with there specific substrates. They accelerate the chemical reactions by several folds.

**Commonly used enzymes in animal nutrition:**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase</td>
<td>Cellulose</td>
</tr>
<tr>
<td>Pentosanase</td>
<td>Pentosans</td>
</tr>
<tr>
<td>Beta glucanase</td>
<td>beta-glucans</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Pectins</td>
</tr>
<tr>
<td>Amylase</td>
<td>Resistant starch</td>
</tr>
<tr>
<td>Protease</td>
<td>Proteins</td>
</tr>
<tr>
<td>Alpha-galactosidase</td>
<td>Oligosaccharides</td>
</tr>
<tr>
<td>Phytase</td>
<td>Phytic acid</td>
</tr>
<tr>
<td>Tannase</td>
<td>Tannins</td>
</tr>
</tbody>
</table>

**2.9.1 Protein degrading enzymes**

Vegetable proteins contain various antinutritional factors. Most legumes contain lectins and protease inhibitors. Protein inhibitors can cause a reduction in chymotrypsin activity and impair digestion, while lectins can damage gut wall, impair immune response and increase endogenous nitrogen loss. Protease works by hydrolyzing proteins or peptides, thus improving protein digestibility-Thorpe and Beal (2001).

**2.9.2 Sources of feed enzymes**

Various fungi, bacteria and to some extent yeast are employed in enzyme production. It is essential for these microorganisms to produce enzymes as they sustain their own viability by producing enzymes to breakdown substrates for further metabolism. Fungi represent largest group among enzyme producing microorganisms, the most important genera are Aspergillus sps, Trichoderma sps,
Penicillium sps and Hemicelia sps. Among bacteria, Bacillus subtilis and Bacillus licheniformis.

### 2.9.3 Effect of enzymes on nutrient digestibility.

The effect of enzymes in improving the apparent metabolizable energy (AME) in poultry, fed corn-soy based diets has been demonstrated in few recent studies in broilers by Pack and Bedford, (1997), Bhat (1998), Zanella et al., (1999), Graham et al., (2002), Kocher et al., 2002

Overall, the addition of enzymes to broiler diets based on corn–soy increased the apparent metabolizable energy by 0.52 to 11.9 per cent.

Nagalakshmi and Devegowda (1992) and Rajeshwara rao and Devegowda (1996) reported an improved digestibility of neutral detergent fiber and acid detergent fiber with the supplementation of enzyme complex containing the activities of cellulase, hemicellulase, beta-glucanase and xylanase, amylase protease respectively, in the broiler diets. Improvements in digestibility have been reported in most studies, the effects of enzyme on protein utilization in broiler fed corn-soy diet has been examined by Kocher et al., (2002), Raghavendra (2004) reported increased feed conversion efficiency with higher levels of deoiled rice bran in laying hen. As Kocher et al., similar observation was observed by Ramesh and Devegowda (2005).

In general, the improvement in nitrogen digestibility with addition of enzyme in poultry fed corn–soy diet, ranging from 1.5 to 9.5 per cent.

### 2.9.4 Broiler meat cholesterol

Cholesterol is a fat like steroid alcohol with a chemical structure C_{27}H_{45}-OH. It is commonly found in foodstuffs of animal origin like milk, meat and egg. Cholesterol is a normal constituent of blood nervous tissue and cell membrane and does not cause any harm when present in normal amount. It is present in the bile, blood, brain tissue, liver, kidney and the adrenal glands, steroids are complex fat-soluble molecules with four-fused ring nucleus called ‘cyclopentanoperhydrophenanthrene nucleus. The two different types of cholesterol existing in the blood are low density lipoprotein (LDL) (bad cholesterol) and high density lipoprotein (HDL) (good cholesterol). Cholesterol is most highly decorated small molecule in biology; so far 13 Nobel prizes have been awarded to the
scientists who devoted major part of their career to cholesterol. Ever since, it was isolated from gall stones; cholesterol has exerted an almost hypnotic fascination for scientists from the most diverse area and medicine (Jain, 2003).

2.9.4. A Biological significance of cholesterol

Cholesterol is an important component in biological system

- Cholesterol is the precursor for the synthesis of vitamin-D, which is needed for proper calcification of bones and teeth.

- Cholesterol is part of the cell membrane in the body including nerve cells.

- It is precursor of bile acids and is essential for the synthesis of steroid hormones (adrenals and gonads) that are vital for maintaining proper metabolism in the body and reproductive functions.

- Cholesterol, when an unoxidized guard the cell membrane phospholipids from free radical damage and protects it’s against atherosclerosis, cancer and other free radical attack (Srinivasaiah, 1998).

- Garlic (*Allium sativium*) is widely distributed all over the world. In the past two decades, particular attention has been focused on its cholesterol lowering activity as reported by Rajashekara Reddy *et al.*, (1991).The cholesterol content of the edible muscle tissue of broiler chickens can be reduced approximately by 25 per cent after feeding a supra normal level of copper for 42 days without altering growth weight of the chickens or substantially increasing the copper content of edible meat (Ramgi *et al.*, 1995). According to Katti *et al.*, (1996) Chitosans is a well-known deacetylated derivative of chitin present in silkwormpupae meal with biochemical property of hypocholesteremic effect.

   Konjufca *et al.*, (1997), observed that the supplementation of copper at either 63 or 180 mg/kg diet as cupric citrate or cupric in Reduction of cholesterol content in poultry meat is one of the better approaches to control dietary cholesterol intake. Konjufca *et al.*, (1997) reported feeding dietary garlic or
copper for 21 days reduces cholesterol levels of broiler meat without altering growth rate of the chickens or feed efficiency. Premkumar et al., (2001), reported that the dietary supplementation of copper and garlic individually and in combination significantly reduced serum total cholesterol and breast meat cholesterol by 23.5 per cent in broilers at market age of seven weeks. There are several cholesterol reducing agents such as copper, garlic, linseed oil and chitosan. Amaranthus plant, lovastatin and ketoisocapric acid. Garlic contains active principles like allium, allylic sulphide, which lowers the low-density lipoprotein (LDL) cholesterol and act as anti-carcinogenic (Narahari et al., 2004). Prakash and Munegowda (2007) reported that supplementation of Abana \textsuperscript{tm} garlic paste at different levels individually and in combination reduced the meat cholesterol significantly as compared to control diet.

2.10 Organoleptic evaluation

Chilever (1970) studied that aromatizing compounds like Zurathiols or Thiopenethiols are suitable for beef soups, chicken soups, chicken broths and meat sauces and balls. Flament et al., (1978), analyzed raw meat extract and commercial beef extract and identified characteristics aroma components. They also detailed extract preparation fraction and analysis procedures. Hammer (1980) discussed the basic principles of sensory analysis methods suitable for use in meat industry with reference to selection and training of test personnel, detection, identification, saturation and discrimination of thresh old values for organoleptic characteristics and sensory analysis of individual samples, descriptive tests profile and dilution profile tests and qualitative evaluation using a scale. Kodkol et al., (1980) found that organoleptic evaluation of concentrated meat gravies canned were acceptable by the panelists after one year of storage. Touralle (1980), considered some aspects of sensory evaluation of meat and meat products. The aspects include, sensory perception, basic principles of sensory evaluation, objective versus subjective evaluation, selection and training personnel, preparation and standardization of meat.