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BIODIVERSITY OF COMMERCIALLY IMPORTANT FRESHWATER PRAWNS IN INDIA WITH EMPHASIS ON THEIR AQUACULTURE POTENTIALS

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ABSTRACT

Nearly 60 species of the freshwater prawns (size range 4.2-38 cm) recorded from the Indian subcontinent, *Macrobrachium rosenbergii* is the most preferred species due to its suitability for aquaculture on account of its fast growth rate, omnivorous feeding habit, hardy nature, compatibility for polyculture, resistance to certain diseases, unique appearance and high prices in domestic as well as in international markets. In natural system, it attains a size of 30-35 cm with 400-450 gm weight thus being the largest prawn available for culture. It grows well in almost all freshwater and low-saline water bodies such as lakes, rivers, swamps, irrigation ditches, canals, ponds and small dams. While cultured in earthen ponds, the ready marketing size of 70-80 gm is obtained over a period of 8-10 months under the tropical climate. Polyculture with compatible species of carps facilitate better utilization of pond resources and also control excessive growth of algae and zooplankton. The grass carp, silver carp, catla, rohu, milkfish and green chromids can be used for polyculture with scampi, however, bottom feeders like the mrigal, common carp and tilapia are not advisable as they compete for food and space.

Key words: *Macrobrachium rosenbergii, polyculture, organic aquaculture.*

Because of its universal appeal, unique taste and low fat content, prawns are fast becoming a popular food item among the young and olds, especially in Japan, United Kingdom, United States, Hong Kong, Singapore and several other countries (New and Valenti, 2000). In all these places, the demand for prawns is increasing day-by-day and the supply can hardly be met (Kutty, 2005). Japan and United States have the biggest frozen prawn markets and these two alone account for about eighty percent of the total world prawn consumption (Kutty, 2005; Nair *et al.*, 2007). Prawn catch in the sea and other traditional natural resources has been stagnating for the last several years and at certain places even fast declining due to many factors which deeply affected it. As such, any further increase to meet the widening supply and demand gap can be achieved only through aquaculture (Kutty, 2005; New *et al.*, 2008). Therefore, a world aquaculture race for prawn culture has been initiated (Upadhyay *et al.*, 2006; Nair *et al.*, 2007; Raju *et al.*, 2009; Marques and Moraes-Valenti, 2012). During the past five years, the global freshwater prawn production registered more than 12 folds increase from 35,573 tonnes in 1995 to 458,000 tonnes valued above US$ 1.8 billion in 2007 in which *Macrobrachium rosenbergii* contributed around 244,000 tonnes (Raju *et al.*, 2009). Between 1999-2003, the annual increase of farmed *M. rosenbergii* production in India was about 80% with production touching 30,450 tonnes in 2002-2003 (Kutty, 2005). As such, India is the second (after China), largest contributor of freshwater prawn to the world markets followed by Thailand, Bangladesh, Taiwan and Vietnam (Balamurgan *et al.*, 2004; Raju *et al.*, 2009). The basic method of prawn culture is almost similar in

every country, the only difference being the variety of prawn each country tends to produce.

Currently, prawn culture technology has advanced so fast that it is now considered a relatively new, progressive and high profitable industry. Because of its high unit value and ever-increasing demand in the world market, prawn farming is found to be one of the most profitable enterprises of the day with a net return of more than Rs.1.5 lakh/ha/year. In India, culture of freshwater prawn is prevalent in about 34,630 ha area with cumulative production to the tune of 30,450 tonnes (Sakthivel, 2003; Balamurgan *et al.*, 2004). During the year 1997-98, approximately 66,000 tonnes of frozen prawns were exported from our country fetching foreign exchange worth Rs. 3,112 crores. Aquaculture production of prawn contributes to 42% by quantity and 68% by value to the total prawn exports worth US$ 579 million from India. However, the quantity of scampy exported from this country was 10,380 tonnes worth Rs. 447 crores during 2002-2003 (Sakthivel, 2003; Nair *et al.*, 2007).

Prawns belong to the freshwater egg-bearing Family Palaemonidae of which *Macrobrachium* is popularly cultured Genus or the marine, non-egg-bearing Family Penaeidae. The United Nations Food and Agricultural Organizations (FAO) adopted the convention of referring all palaemonids as prawns and all penaeids as shrimps (Apud *et al.*, 1985). However, there is no clear-cut distinction between the terms shrimp and prawn and they are being used interchangeably with emphasis on one or the other in different parts of the world. Among the shrimps, *Penaeus monodon* (tiger prawn), *P. indicus* (white prawn) and *P. merguiensis* (banana prawn) *etc* are a few commercially important species. They require sea water or brackishwater for their growth. Nearly 60 species of the freshwater prawns (size range 4.2-38 cm) have been recorded from the Indian subcontinent which include - *Macrobrachium aemulum, M. altifrons altifrons, M. altifrons ranjhai, M assamense assamense, M. assamense peninsulare, M. australe, M. banjarae, M. birmanicum, M. canarae, M. cavernicola, M. dayanum, M. divakarani, M. dolicodactylus, M. elatum, M. equidens, M. gangeticum, M. gurudeve, M. hendersodayanam, M. hendersoni hendersoni, M. hendersoni cacharense, M. hendersoni platyrostres, M. hainaense, M. idae, M. idella idella, M. idella georgi, M. indicum, M. javanicum, M. jayasree, M. johnsoni, M. josephi, M. kempi, M. kistense, M. kulsiense, M. kunjuramani, M. lamarrei, M. lamarrei lamarroides, M. latimanus, M. malcolmsoni, M. manipuresnse, M. mirabile, M. naso, M. nobilii, M. nowaehollandiae, M. ornatus, M. peguense, M. rogersi, M. rosembergii, M. rude, M. sankoli, M. scabriculum, M. siwalikense, M. sulcatus, M tiwarii, M. unicarnatakae, M. veliense* and *M. vellosimanus* (Jayachandran, 2001; Jayachandren and Indira, 2010). Among these freshwater prawns, *Macrobrachium rosenbergii* (the giant long-legged river prawn), *M. malcomsonii, M. choprai (M. gangeticum), M. dayanum and M lamarrei, M. villosimanus, M. josephi, M. idella idella, M. idella georgi, M. rude, M. equidens, M. scabriculum, M. lanchesteri, M. sulcatus, M. mirabilis, M. kistense and M. latimanus* are commercially important species (Kanaujia, 2003; Nair *et al.*, 2007). Of them, the first three species are suitable for aquaculture in India. They require freshwater (sweet water) or low-saline water for their growth (Rao and Tripathi, 1993; Kanaujia, 2003). Species of the freshwater prawns of genus *Macrobrachium* are distributed throughout the tropical and subtropical zones of the world. They are found in most inland water areas including lakes, rivers, swamps, irrigation ditches, canals and ponds as well as estuaries. Most species require brackishwater in the initial stages of their life-cycle and therefore, they are found in water that is directly or indirectly connected with the sea. However, some complete their life-cycle in inland saline and freshwater lakes (Rao and Tripathi, 1993; Kanaujia, 2003).

Macrobrachium rosenbergii is a crustacean with exoskeleton or shell. The body of prawn is divided into head, abdomen and tail. There are five pairs of walking legs. The first pair is used for putting feed into the mouth. The second pair is much larger than the others and ends in pronounced claws. It is used for self-defense and catching food. The rostrum develops at the tip of the head. Dorsal and ventral teeth numbers are 12-15 and 8-14, respectively. There are five pairs of swimming legs at the abdomen with one pair at each abdominal segment, except the last one. The tail part is composed of two uropods and one telson. The head of mature female and its second walking legs are much smaller than the adult male. The genital pores are at the base of third walking legs, the pleura of the abdomen are longer and the abdomen itself is broader. The pleura form a brood chamber in which the eggs are carried during laying and hatching. A ripe or ovigerous female can easily be detected because the ovaries can be seen as large orange coloured masses occupying a large portion of the dorsal and lateral parts of cephalothorax. Like other crustaceans, all freshwater prawns have to regularly cast their exoskeleton or shell in order to grow. This process is referred to as moulting and is accompanied by a sudden increase in size and weight. The number of moults and the duration of intermoult are not fixed and depend on the environment, particularly temperature and availability of food. *Macrobrachium* spp. has a smooth round dorsal surface to the abdomen while penaeids have a simple or complex ridge at the dorsal apex of the abdomen. Moreover, the second pleuron of the abdomen (or tail) of the species overlaps both the first and third pleura. In penaeids, the second pleuron overlaps the third pleuron only and is itself overlapped by the first (D'Abramo and Brunson, 1996). *Macrobrachium rosenbergii* is indigenous to South and Southeast Asia, Northern Oceania and in the Western Pacific islands. As this species is the most favoured for commercial farming, it has been introduced to more countries covering almost every continent (Nair *et al.*, 2007; Marques and Moraes-Valenti, 2012). *M. rosenbergii* is now farmed in considerable quantity in many countries including Hawaii, Honduras, Mauritius, Taiwan and Thailand and the farms are now being established in many other countries including India, Costa Rica, Indonesia, Israel, Malaysia, Mexico, the Philippines, Zimbabwe *etc* (D'Abramo and Brunson, 1996).

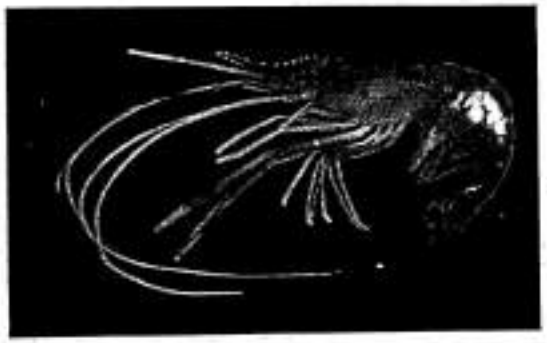
Of the total global production of 8,06,260 tonnes of cultured crustaceans during the year 1991, marine prawns contributed 90.5% while contribution of freshwater prawns was only 4.1% (New, 1994). The percentage sharing of freshwater prawns in the total 6,23,709 tonnes production of prawns in Asia during 1991 was only 5% compared to 95% that of marine prawns. Global production of formed *M. rosenbergii* was estimated to be 33,297 tonnes in the year 1991 and 1,19,000 tonnes during 2000 (FAO, 2002; Kutty, 2003). Over 93% of them are produced in Asia, 52% of Asian freshwater prawn production was from Taiwan while Thailand and Vietnam contributed 24% and 23%, respectively. The other Asian countries where freshwater prawn culture is being practiced includes India, Japan, Myanmar, Brunei Darussalem, Combodia, China, Indonesia, Iran, Nepal, Pakistan, Bangladesh, the Philippines and Saudi Arabia (Phuong *et al.*, 2003; Taynmen, 2003; Yoonpundh *et al.*, 2003; Hossain, 2003). Though FAO has given the production level of over 1,28,000 tonnes of *M. rosenbergii* for China during 2001 but they claim to have achieved the total production of cultures freshwater prawns (*Macrobrachium* spp.) over 21,000 tonnes during this period (Weimin, 2003; Raju *et al.*, 2009).

Outside Asia, the two South American countries Ecuador and Brazil were the next major contributors to the global production of freshwater prawns during 1991. In Brazil, there are more than 600 grow-out culture farms and with productivity varying from 1,000-4,500 kg/ha/year total production of 400 mt was realized (Valenti, 2003). Colombia, Guyanne, Peru, Surinam and Venezuela are few other South American countries where freshwater prawn farming is being practiced (New and Valenti, 2000). Among the North America and Caribbean countries, contribution of Mexico, Hawaii and Commonwealth of Puerto Rico in the global production of freshwater prawns during 1991 has been important. The Dominican Republic, Jamaica, Guadeloupe and Martinique in the Caribbean are significant producers of the freshwater prawns. Costa Rica, El Salvador, Guatemala, Honduras, Panama and Lucia are few

other countries were freshwater prawn farming is gaining importance (Nambudiri, 2003). Compared to other parts of the world, relatively insignificant quantities of freshwater prawns are cultured in South Africa. Mauritius and Zimbabwe are the major producers in South Africa, other countries being La Reunion and Malawi. Production of *M. rosenbergii* in the Pacific region is very small. Apart from French Islands, only Fiji, Guam and Solomon Islands have reported the production of freshwater prawns (New and Valenti, 2000). In India, only 1,49,591 ha has so far been brought under prawn farming yielding about 80,000 tonnes of prawn annually with maximum share of *P. monodon*. The quantity of freshwater prawns produced during 1991 was around 10,000 tonnes. There is enthusiastic interest amongst farmers and entrepreneurs for freshwater prawn aquaculture in India, especially from Andhra Pradesh which contributes to 88.6% of total freshwater prawn production during 2002-2003 and more and more new farms are being developed day-by-day for the purpose (Kutty, 2003). However, at present quantitywise it has little contribution in total export of fishes and fishery products from our country.

Freshwater Prawn Aquaculture in India

Development of aquaculture in India is centred around prawn culture due to its high unit value realization and ever-expanding export demand. Scientific culture of marine prawn started in India during eighties and by mid-nineties, more than one lakh ha. area was brought under culture. However, the rapid growth of the marine prawn farming industry halted suddenly in 1994-95 along the East coast and in 1995-96 along the West coast. The collapse of the industry was attributed mainly to environmental and health problems resulting in the outbreak of the diseases (Sakthivel, 2001). Subsequently, marine prawn farming industry suffered yet another setback due to judgment given by Honourable Supreme Court of India during 1996 banning setting up of prawn culture ponds within coastal regulation zone (CRZ). Consequently, a great interest has been developed in India for freshwater prawn aquaculture and during the past seven-eight years several new farms have been developed. More and more prawn farmers of the country are turning to freshwater prawn aquaculture to overcome the setback in marine prawn farming (Sakthivel, 2003).



India has vast potential for commercial farming of both marine as well as freshwater prawns and possesses one of the richest resources for freshwater prawn aquaculture in the world. On account of its ideal climatic conditions, it can be regarded as the "sleeping giant" for freshwater prawn farming in Asia. While around 1.2 million ha of coastal area located in and around backwaters, estuaries and other brackishwater bodies provide potential sites for marine prawn farming, a vast portion of another several million ha area in and around the close vicinity of 2.25 million ha of ponds and tanks, 1.30 million ha of beels, jheels and derelict waters, 2.09 million ha lakes and reservoirs and also 0.12 million km of canals and channels as well as a portion of about 2.30 million ha of paddy fields can be scientifically developed for commercial exploitation of freshwater prawn through aquaculture or culture-based capture (Jhingram, 1991). Of the 1.9 million ha available freshwate ponds, if 0.3 million ha is used for prawn culture, the production of scampy can be raised to 1,50,00 tonnes worth Rs. 3,000 crores (Sakthivel, 2003).

Of the 200 species of freshwater prawns, *M. rosenbergii*, commonly called "scampi" is the mos preferred species due to its suitability for aquacultur on account of its fast growth rate, omnivorous feedin habit, hardy nature, compatibility for polyculture resistance to certain diseases, unique appearance an high prices in domestic as well as in internationa markets. In natural system, it attains a size of 30-3 cm with 400-450 gm weight thus being the large prawn available for culture. It grows well in almost a freshwater and low-saline water bodies such as lake rivers, swamps, irrigation ditches, canals, ponds an

small dams. However, while cultured in earthen ponds, the ready marketing size of 70-80 gm is obtained over a culture period of 8-10 months under the tropical climate. Polyculture with compatible species of carps will facilitate better utilization of pond resources and also control excessive growth of algae and zooplankton. The grass carp, silver carp, catla, rohu, milkfish (*Chanos chanos*) and green chromid (*Etroplus suratensis*) can be used for polyculture with scampi. However, bottom feeders like mrigal, common carp and tilapia are not advisable for polyculture with scampi as they are competitors for food and space (Jose, 2003; Radheyshyam, 2009).

Scientific commercial farming of scampi has just started in our country. Since the net return from such farming is much more than normal fish farming, several new farms are being developed for monoculture of scampi or mixed culture with other fish species, especially in the states of Andhra Pradesh, West Bengal, Kerala, Orissa, Maharashtra, Punjab, Haryana and Gujarat (Upadhyay, 1995; Vasudevappa, 2001; Sakthivel, 2003; Singh, 2003). Apart from development of new farms, several existing fish farms are now being used for monoculture or mixed culture of *M. rosenbergii*. Most of the farms in West Bengal, Orissa and Gujarat use seed collected from the wild whereas those in Andhra Pradesh, Tamil Nadu, Kerala and Maharashtra mainly use hatchery produced seed for culture. However, a major constraint in the development of this aquaculture in our country has been the scarcity of seed (Mohanta, 2000). Hatchery technology for production of *M. rosenbergii* seed has been developed and about 71 hatcheries have been setup in India with a production capacity of more than 13 million seeds to overcome this problem (Bojan, 2003). Even though site conditions and environmental factors being ideal for prawn aquaculture in the states like Uttar Pradesh, Bihar and Madhya Pradesh, its importance is yet to be demonstrated and popularized in these areas (Janaki Ram and Pandey, 2003; Bojan and Viswakumar, 2003; Sultan, 2003). Keeping in mind the vast potential for freshwater prawn culture in our country, even 10% of its utilization on scientific lines can earn daily bread for millions of people besides bringing billion dollars to the nation.

Like other forms of aquaculture, prawn farming is limited by environmental constraints. *M. rosenbergii* is amenable to extensive, modified extensive as well as semi-intensive culture. However, it cannot be reared as intensively as marine prawns. Considering the vast potential areas available in our country for cultures and with a view to ensure long sustaining yield, better economics and eco-friendly practice, it is advisable to adopt to extensive or modified extensive farming system. The over-intensification in marine prawn culture has already proved to be disastrous and invited a lot of criticisms in several corners including Taiwan, Thailand, China, India and other countries. However, farming of *M. rosenbergii* is more environmentally sustainable because of its lower grow-out intensity. Moreover, in contrast to marine prawn culture, it does not require seawater except in hatcheries or coastal sites. Even hatcheries can be operated inland by diluting transported seawater, brine or artificial sea salt (Mishra et. al., 2011). The hatchery period is twice as long as that for marine shrimp (New, 1994; Kanaujia, 2006).

Culture of Freshwater Giant Prawn

Culture operation of *M. rosenbergii* in artificial ponds can be divided into following five steps: (i) pond preparation, (ii) transportation of seed to the farming sites, acclimatization and stocking, (iv) culture techniques, (v) water quality and feed management and (vi) harvesting.

Pond preparation: Pond preparation includes drying, liming, ploughing of pond bed, application of mahua oil-cake or other fish killer to eradicate predators from the pond, application of lime and manure (raw cow dung *etc*) and fertilizers (urea and single-super-phosphate or NPK). Ponds should be dried up till they crack. Thereafter, ponds are limed and tilled. Application of lime adjusts soil and water pH, sterilizes pond bottom, maintains optimum alkalinity, helps decompose organic matter and kills predators or other undesirable aquatic organisms living at pond bottom.

This improves pond condition and increases production. Various types of compounds can be used for liming during pond preparation. However, the application rate varies with soil pH.

Then water should be filled into the pond to an average depth of nearly 40 cm and subsequently tea seed cake powder @ 150-200 kg/ha be added to kill predators and other aquatic organisms. The pond should be further enriched with organic manures such as dried chicken manure @ 150-22 kg/ha or raw cow dung @ 500-1,000 kg/ha and fertilizers such as urea and single super phosphate @ 50-70 kg/ha. The pond should be left for 4-5 days to provide time for the growth of natural food. When colour of the pond water turns green or brown, more water is filled in until the desired depth of 1.25 meter is attained. Now the pond is ready for stocking.

Transportation of seed to sites and stocking: Two weeks old post-larvae (PL) of *M. rosenbergii* produced in a hatchery or collected from natural resources such as river *etc* are transported to the farming site in oxygenated polythene bags packed in insulated boxes made of card boards and thermocol. Prawn seeds can be transported for 18-20 hours at a packing density of 250 PL/litre. Before releasing the prawn seeds into the pond, they are properly acclimatized for a period of 1-2 hours by keeping the polythene bags (with seeds) open and then kept in the pond water adjacent to bundhs slowly sprinkling water onto them. The acclimatization should be normally done in the morning so that the water temperature fluctuation is minimized. Sample crotons should be counted for stock estimation. The seeds are then released into the ponds at the desired stocking density. The post-larvae (PL) obtained from hatchery could be stocked into the culture ponds directly after acclimatization. However, it is advisable to rear them in small nursery tanks for a period of one month before transferring into the culture ponds. This ensures predictable percentage of survival and shortens the grow-out phase. An initial density of 50,000 number of one month old prawn PL /ha, 70 gm size is easily achievable in a growing period of 8 months. Some of the farms in Thailand and Hawaii stocking of as much as 2,00,000 seeds/ha is in practice followed with cull-harvesting resulting in higher production but the size at harvest is reduced.

Culture techniques: The two types of culture techniques being adopted for prawn culture are - (i) continuous culture with cull harvesting and (ii) batch culture and batch harvesting or drain harvesting. Continuous culture with cull harvesting or repeated culling of larger prawns is widely adopted in Thailand and Hawaii. It consists of stocking the ponds, usually once a year or sometimes 4-6 times a year at high stocking densities and after about 5-7 months, culling of marketable-sized prawns at regular intervals. The ponds are not drained out but the larger ponds are fished out by seining. Following this system, a production upto 276 kg/ha/month has been reported in Hawaii which works out to be 3,312 kg/ha/year. The yield varies from 2,500-5,000 kg/ha/year (New and Singholka, 1985; Upadhyay, 1995; Nair and Salin, 2003). The other technique consists of batch culture and batch harvesting or drain harvesting. It involves stocking the ponds at the optimum level for maximum rate of growth and harvesting the whole crop, possibly by draining the ponds.

In Thailand, most farmers adopt a combination of these two techniques. About 5 months after the post-larvae stocked, cull harvesting commences to be repeated once every month until the eight month when the pond is drained completely and the prawns harvested. The pond is again prepared and restocked when water supply is available again. An estimated production of 3,800-4,700 kg/ha/year has been reported with this culture practice under semi-intensive prawn farming. In an experimental tank, a production rate of 3,300 kg/ha/year has already been achieved in India. Under agro-climatic conditions of Uttar Pradesh, an average production of freshwater giant prawn 800-1,000 kg/ha/6 months under monoculture operation has been realized (Sultan, 2003).

Water quality and feed management: The main purpose of water exchange from the aquaculture pond is to maintain the water quality. It also stimulates moulting of the prawn resulting in acceleration of growth and production. Depending upon various physico-chemical parameters of the pond water such as dissolved oxygen content, transparency, algae density, stocking density and stage of culture, the amount of water to be exchanged from any aquaculture pond will vary considerably. For a prawn culture pond with initial stocking density of 5-7/m², the average daily water exchange requirement may be taken as 10% of the total water volume of the ponds. The pumping capacity should be sufficient enough to meet this requirement. For a higher stocking density of 12-15/m², the average daily water exchange may be 25-30% of the total pond water volume. Usually water exchange starts after one month of initial stocking. In the beginning, it may be 5% only and reaches to maximum towards harvest. If the dissolved oxygen content of the pond water body is lower than 3 ppm in the morning or water transparency is less than 30 cm, percentage of water exchange requirements from the ponds will be more than usual. pH of the pond water is maintained to be around 8. During culture period, 200-300 kg lime/ha may be applied every week from second week onwards for getting better result. It is advisable to use agricultural lime (calcium-magnesium carbonate) during the culture period. For a pond having higher stocking density of prawns, use of paddle wheel aerators becomes essential to check depletion of dissolved oxygen level in the pond. The water quality parameters like temperature, pH and dissolved oxygen levels should be monitored continuously.

Diet of *M. rosenbergii* consists of aquatic insects and larvae, small molluscs, fish and offals of other animals, algae, grains including rice seeds and fruits. They accept compounded feeds, chopped butchery wastes, tapioca, oil-cakes *etc* and occasionally may turn cannibalistic too. They relish live organisms and therefore manuring the ponds to increase benthic fauna is advantageous. For a prawn farm with targeted production of 1-1.5 tonnes/ha/year, mostly farm prepared feed is used. However, a scientific prawn farm with comparatively high targeted production of 2 tonnes/ha/year and above, application of pelletized feed containing high protein percentage is essential (Raju, 2003). Food is usually spread around the periphery of the pond or presented in predetermined areas a few metres apart. The intention is to observe how much feed has been consumed. The feed ratio will have to be increased or decreased according to the extent of consumption by the prawns. They may be fed once a day at 4-5 p m, five days out of 7, with pelletized feed 1 mm in diameter in nursery ponds and 4 mm in diameters in grow-out ponds. The daily ration is calculated from the estimated total weight of prawn then a theoretical daily feeding rate is controlled by the observation of remains from the dike or underwater feed trays. The observation of prawns and measurement of growth can be done through periodic seining (every second week) of samples including few hundred prawns from two different locations of the pond. The days when any moulting occurs, no feed is given. The theoretical daily feeding rate may be assumed to be 10% of body weight of prawns at initial stage. This may be gradually reduced to nearly 2% towards harvest. Usually during the initial two weeks of stocking, supplementary or pelletized feed is not given as prawns can eat natural food. Feed conversion ratio (FCR) of a good pelletized feed is usually 2:1 to 1.5:1 (New, 1994; New and Valenti, 2000; Mohanta, 2000; Mitra *et al*., 2005).

Harvesting: In cull harvesting usually bottom seining is done and the first harvest takes place 5-7 months after initial stocking. In batch harvesting usually the pond is drained. Prawns are caught by multiple seining followed by hand picking. The final draining of pond is made through a net that retains the prawns. In all cases, harvesting operations should take place in the early morning hours when it is cooler. Head on prawns are transported to the processing plant after proper icing. Depending upon existing infrastructure facilities, location of the sites, distance from the water source, topography and various other parameters as well as

type of technology adopted for culture, the development as well as operating cost of one scampy farm may vary. The commercial scientific farming of scampi has become popular in several parts of the world. Much of the potential for prawn culture has not yet been realized. This form of aquaculture is particularly appropriate for small-scale units, though to exploit export markets, produced groups or marketing organizations will be essential. Substantial expansion of freshwater prawn farming is expected, especially in the Asian farm production by the year 2020 (New, 1994; Bojan and Viswakumar, 2003; Sakthivel, 2003).

Organic Scampy Farming in India

Organic farming systems rely on the ecologically-based practices including culture and biological pest management completely excluding the use of synthetic chemicals in crop production and prohibit the applications of antibiotics as well as hormones in livestock production. The preference of consumers demanding for organic products is reflected in the increase in organic commodities found in the market places, especially in the United States and European Union (EU). Thus enhanced demand for such food products may lead to the increased profitability for all concerned (Bergleiter *et al*., 2009). Organic aquaculture is a new concept for this country (Purushan, 2008; Kumar and Pandey, 2010). It is a holistic production management system which may play a pivotal role in development of aquaculture as well as fish and shellfish diversity conservation. Our traditional (extensive) and semi-intensive prawn farming practices continued to sustain the aquatic environment as well as livelihood of fish farmers. Organic aquaculture is yet to find a place in the farming systems of this country. Keeping the huge potential of selling aquaculture products in markets of European Union and USA, the Marine Products Export Development Authority (MPEDA) (Ministry of Commerce, Government of India), Cochin has initiated the Indian Organic Aquaculture Project (IOAP) on organic black tiger prawn (*P. monodon*) and scampi (*M. rosenbergii*) farming in Kerala and Andhra Pradesh in January 2007 in technical and consultancy collaboration with Swiss Import Promotion Programme ((SIPPO). M/S Rosen Fishery Hatchery, Trichur has produced 11.50 lakh organic scampy seeds and supplied the same to Kerala (3.4 lakh) and Andhra Pradesh (8.1 lakh) for organic freshwater giant prawn aquaculture. Harvest of the first organic scampy was done on 01.11.2008 in 20 ha spread over four farms in Kuttanad of Alappuzha district of Kerala. Buyers were from Germany, exporters, officials from SIPPO and Naturland Association (Germany). The organic prawns were sold @ 350-500/kg. With this, India has also embarked on the path of organic aquaculture which will be expanding with the active support of MPEDA. The industrialized and developed countries of the West where affluence, education and consumer awareness are quite high remain as the main destinations of organic prawn products.

Aquaculture of Minor Species of Prawns

Though *M. rosenbergii* is the fastest growing natantian but the success of small-sized *M. nipponense* for aquaculture in China has opened the avenues for the entry of other minor species for aquaculture production as well as diversification (Kutty, 2003). In African countries, trials are being conducted to introduce *M. carcinus*, *M. amazonicum*, *M. acanthurus* and *M. vollenhovenii* in freshwater aquaculture. It is interesting to note that *M. malcolmsonii* accounts for more than 10% of artisanal aquaculture and an yield of 327-805 kg/ha/year under monoculture with wild seed and 880-1,130 kg/ha/year with hatchery-produced seed has been achieved (Kanaujia *et al*., 1997). Polyculture of this species with fish is commonly practiced in Orissa due to natural availability of seed from rivers. In this system, compatible carp species such as *Catla catla* (surface feeder), *Labeo rohita* (column feeder) and grass carp, *Ctenopharyngodon idella* (plant eater) and silver carp, *Hypophthalmichthys molitrix* (phytoplankton feeder) are cultured while bottom feeder carps like *Cirrhinus mrigala* and *Cyprinus carpio* are not used (Kanaujia *et al*., 1997; Radheyshyam, 2009). Under prawn polyculture

operations, 170-327 kg/ha/year prawn and 2,084 kg/ha/year fish has been recorded (Kanaujia, 2006). The recent development in seed production, hatchery management, larval rearing and domestication of *M. malcolmsonii* and *M. gangeticum* (*M. birmanicum chpvari*) will go a long way in diversification of freshwater prawn culture in India (Kutty, 2005; Kanaujia, 2006; Radheyshyam, 2009; Mishra *et al.*, 2011).

REFERENCES

Apud, F.; Primavera, J.H. and Torres, P.L. Jr. (1985). *Farming of Prawns and Shrimps*. Aquaculture Extension Manual No.5. SEAFDEC, Philippines.

Balamurgan, P.; Mariappan, P. and Balasundaram, C. (2004). Impacts of monosex *Macrobrachium* culture on the future of seed availability in India. *Aaacult. Asia*, 9 (2): 15-16.

Bergleiter, S.; Berner, N.; Censkowsky, U. and Julia-Camprodon, G. (2009). *Organic Aquaculture 2009: Production and Markets*. Naturland-Association for Organic Farming & Organic Services, Germany.

Bojan, J. (2003). Status of scampi farming in India. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 14.

Bojan, J. and Viswakumar, M. (2003). Role of MPEDA in promotion of scampi culture in India. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). Kerala Agricultural University, Cochin. pp. 33-35.

D'Abramo, L.R. and Brunson, M.W. (1996). *Biology and Life-History of Freshwater Prawns*. SRAC Pub. No. 483. Southern Regional Aquaculture Centre, Mississippi (USA).

FAO (2002). *Fishery Statistics-Aquaculture Production 2000*. Food and Agriculture Organizations, Rome.

Hossain, M.I. (2003). Freshwater prawn status in Bangladesh: its strategy and policy. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p.15.

Janaki Ram, K. and Pandey, A.K. (2003). Status and prospects of aquaculture development with special reference to Uttar Pradesh. In: *Brain Storming Session on Production Status and Potential of Fisheries in U.P.: Constraints and Opportunities* (January 22, 2003). Uttar Pradesh Council of Agricultural Research, Lucknow. pp. 24-38.

Jayachandran, K.V. (2001). *Palaemonid Prawns: Biodiversity, Taxonomy, Biology and Management*. Science Publishers, Enfield & Plymouth.

Jayachandran, K.V. and Indira, B. (2010). Sustainable exploitation of freshwater prawn diversity of India for food and livelihood security with emphasis on planning. *Indian J., Sci. Res.*, 1: 127-132.

Jhingran, V.G. (1991). *Fish and Fisheries of India*. Hindustan Publishing Company, New Delhi.

Jose, S. (2003). Pond culture strategies of giant freshwater prawn-thoughts for tomorrow. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 40-42.

Kumar, R. and A.K. Pandey (2011). Prospects of organic aquaculture in India. In: *Organic Agriculture: Hope of Posterity* (Neeru Bhooshan and Chandrika Prasad, eds.). Uttar Pradesh Council of Agricultural Research, Lucknow. pp. 195-209.

Kanaujia, D.R. (2003). Indian river prawn, *Macrobrachium malcolmsonii* and minor species of commercial importance. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 51-58.

Kanaujia, D.R. (2006). Freshwater prawn breeding and culture. In: *Handbook of Fisheries and Aquaculture* (Ayyappan, S., Jena, J.K., Gopalakrishnan, A. and Pandey, A.K., eds.). Directorate of Information and Publication in Agriculture (ICAR), New Delhi. pp. 293-306.

Kanaujia, D.R.; Mohanty, A.N. and Tripathi, S.D. (1997). Growth and production of Indian river prawn, *Macrobrachium malcolmsonii* (H. Milne Edwards) under pond conditions. *Aquaculture*, 154: 79-85.

Kutty, M.N. (2003). Sustainable aquaculture: a comparative view of freshwater prawn and marine shrimp. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 14-20.

Kutty, M.N. (2005). Towards sustainable freshwater prawn aquaculture-lessons from shrimp farming, with special reference to India. *Aquacult. Res.*, 36: 255-263.

Marques, A.H.L. and Moraes-Valenti, P.M.C. (2012). Current ststus and prospects of farming the giant river prawn, *Macrobrachium rosenbergii* (de Man 1879) and the Amazon river prawn, *Macrobrachium amazonicum* (Heller 1862) in Brazil. *Aquacult. Res.*, 43: 984-992.

Mishra, P.; Kanaujia, D.R.; Bohidar, K. and Pandey, A.K. (2011). Seed production of Gangetic prawn, *Macrobrachium gangeticum* (Bate), in natural seawater and brine solution. *Natl. J. Life Sci.*, 8: 105-112.

Mitra, G.; Mukhopadhyay, P.K. and Chattopadhyay, D.N. (2005). Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming. *Aqua Feeds: Formulation & Beyond*, 2 (1): 17-19.

Mohanta, K.N. (2000). Development of giant freshwater prawn broodstock. *NAGA* (ICLARM), 23 (3): 18-20.

Nair, C.M. and Salin, K.R. (2003). Scampi aquaculture in India-an overview of the techniques and the emerging threats to sustainability. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 25-32.

Nair, C.M.; Nambudiri, D.D.; Jose, S.; Sankaran, T.M.; Jayachandran, K.V. and Salin, K.R. (2007). *Freshwater Prawns: Advances in Biology, Aquaculture and Marketing*. Allied Publishers, New Delhi & Mumbai.

Nambudiri, D.D. (2003). Freshwater prawns. *Curr. Sci.*, 85: 1396-1398.

New, M.B. (1994). Freshwater prawn farming: a review of current research, global status, opportunities and constraints. In: *Proceedings of the Workshop on*

Freshwater Prawn Farming in India (March17-18, 1994). Special Publication No.10.Asian Fisheries Society: Indian Branch, Mangalore. pp. 1-24.

New, M.B. (2003). The role of freshwater prawn in sustainable aquaculture. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 10-13.

New, M.B. (2005). Freshwater prawn farming: global status, recent research and a glance at the future. *Aquacult. Res.*, 36: 21-230.

New, M.B. and Singholka, S. (1985). *Freshwater Prawn Farming-a Manual for the Culture of Macrobrachium rosenbergii.* Food and Agriculture Organization of the United Nations. Fisheries Technical Paper No. 225.

New, M.B. and Valenti, W.C. (2000). *Freshwater Prawn Culture*. Blackwell Science, Oxford, United Kingdom.

New, M.B.; Nair, M.C.; Kutty, M.N.; Salin, K.R. and Nandeesha M.C. (2008). *Macrobrachium: The Culture of Freshwater Prawns*. MacMillan India, Chennai.

Phuong, N.T.; Hai, T.N. and Hien, T.T.T. (2003). Development of giant freshwater prawn (*Macrobrachium rosenbergii*) farming in Vietnam. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 12.

Purushan, K.S. (2008). Organic aquaculture eco-friendly. *Kerala Calling Fisheries*, 1: 92-93.

Radheyshyam (2009). Farming the freshwater prawn, *Macrobrachium malcolmsonii*. *Aquacult. Asia*, 13 (1): 29-32.

Raju, U.K.V. (2003). Scampy farm development in India-some thought for future. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 36-37.

Raju, U.K.V.; Nair, C.M. and Salin, K.R (2009). Freshwater prawn farming in India: falling yields signal need for stock improvement. In: In: *Souvenir: Indian Fish Festival* (July 11-13, 2009). National Fisheries Development Board, Hyderabad. pp. 23-31.

Rao, K.J. and Tripathi, S.D. (1993). *A Manual on Giant Freshwater Prawn Hatchery*. Manual Series No. 2. Central Institute of Freshwater Aquaculture, Bhubaneswar.

Sakthivel, M. (2001). Need for legal framework for sustainable aquaculture. In: *Sustainable Indian Fisheries* (Pandian, T.J., ed.). National Academy of Agricultural Sciences, New Delhi. pp. 225-231.

Sakthivel, M. (2003). Utilization of untapped water resources for scampy culture for a rapid jump in production and export in India. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 21-24.

Singh, H. (2003). Record growth and production of giant freshwater prawn, *Macrobrachium rosenbergii* in Haryana-new possibilities. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 96.

Sultan, S. (2003). Freshwater prawn culture in Uttar Pradesh. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 18.

Tayamen, M.M. (2003). Status of giant freshwater prawn, *Macrobrachium rosenbergii*: programme in the Philippines. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 11.

Upadhyay, A.S. (1995). *Freshwater Prawn Farming-A New Horizon for Rural Development*. Bihar Vikas Foundation, Bombay. 38 p.

Upadhyay, A.S.; Kulkarni, B.G. and Pandey, A.K. (2006). Recent advances in giant freshwater prawn culture in India. In: *Recent Advances in Applied Zoology* (Singh, H.S., Chaubey, A.K. and Bhardwaj, S.K., eds.). Choudhary Charan Singh University, Meerut. pp. 116-132.

Valenti, W.C. (2003). Current status of freshwater prawn culture in Brazil. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 16.

Vasudevappa, C. (2001). Status and scope for enhancing giant freshwater prawn production in India. *In: International Symposium on Fish for Nutritional Security in the 21st Century* (December 4-6, 2001). Central Institute of Fisheries Education, Mumbai. pp. 154-159.

Weimin, M. (2003). Status of freshwater prawn culture in China and future prospects. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 10.

Yoonpundh, R.; Srithong, C.; Yi, Y. and Derun, Y. (2003). Current status of freshwater prawn farming in Thailand with special reference to the backyward hatcheries. In: *International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. p. 9.

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COMMERCIAL PROBIOTIC (PROBLEND) ENHANCES GROWTH AND SURVIVAL IN POST-LARVAE OF *MACROBRACHIUM GANGETICUM*

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ABSTRACT

Post-larvae of the Gagetic prawn, *Macrobrachium gangeticum*, fed a diet supplemented (@ 0.8%) with commercial probiotic (Problend) @ 10% of their body mass for 90 days registered significant ($P < 0.05$) increase in body weight by improving specific growth rate (SGR) and feed conversion ratio (FCR). Survival rate of the larvae in control and test diet was 65% and 85%, respectively.

Key words : Dietary probiotic supplementation, growth, survival, post-larvae, *Macrobrachium gangeticum*.

Prawn catch in the sea and other traditional natural resources has been stagnating for the last several years and at certain places even fast declining due to many factors which deeply affected it. As such, any further increase to meet the widening supply and demand gap can be achieved only through aquaculture (Kutty, 2005; New *et al.*, 2008). Over the last two decades, the shrimp farming has been hampered seriously due to outbreak of white spot disease (WSD) causing significant loss to aquaculture production and foreign exchange earnings (Kutty, 2005; New, 2005; Nair *et al.*, 2007). Freshwater prawn farming is considered as an alternative to shrimp farming. Nearly 60 species of the freshwater prawns (size range 4.2-38 cm) have been recorded from the Indian subcontinent among which *Macrobrachium rosenbergii* (the giant long-legged river prawn), *M. malcomsonii, M. choprai (M. gangeticum), M. dayanum, M lamarrei, M. villosimanus, M. josephi, M. idella idella, M. idella georgi, M. rude, M. equidens, M. scabriculum, M. lanchesteri, M. sulcatus, M. mirabilis, M. kistense and M. latimanus* are commercially important species, the first three are suitable for aquaculture in India (Jayachandran, 2001; Kanaujia, 2003; Jayachandran and Indira, 2010). Among freshwater prawns, *Macrobrachium gangeticum* is the third largest growing species which attain maximum length and weight (male 250 mm and 100 mg; females 200 mm and 75 gm) in the Ganges and Brahmaputra riverine system (Kanaujia *et al.*, 2005). Probiotic is cultured product or live microbial supplement when administered via feed, immersion or by injection in adequate amounts confer a health benefit on the host (Fuller, 1989; Irianto and Austin, 2002; Rengpipat, 2005; Denev *et al.*, 2009; Dharmaraj and Kandasamy, 2010). Probiotics are commonly consumed as part of fermented foods with specially added active live cultures such as in yogurt, soy-yogurt or as dietary supplement (Balcazar *et al*, 2006; Yousefian and Amiri, 2009). The use of probiotics in aquaculture has tremendous scope and glorious future (Chen *et al.*, 1992; Moriarty, 1997; Velmurugan and Rajagopal, 2009). Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics but certain yeasts and bacilli may also be helpful (Vijayakumaran, 2001; Balcazar *et al.*, 2006; Deeseenthum *et al.*, 2007; Yousefian and Amiri, 2009). The use of probiotics to maintain healthy environment and improve production has been advocated but the observations are inconsistent and

warrant further work for beneficial applications in prawn aquaculture (Dalmin *et al.*, 2001; Niruawe and Selvin, 2009; Rahman *et al.*, 2009; Yousefian and Amiri, 2009; Habib, 2010; Ngo and Fotedar, 2010; Nimrat and Vuthiphandchai, 2011). We report the effect of a commercial probiotic (Problend, powder form) on post-larval growth and survival of *M. gangeticum* under hatchery conditions.

MATERIALS AND METHODS

The study was carried out at Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar (Orissa) during June-August 2010 in triplicate in six number of 500L FRP tanks. Stocking was done with uniform sized post-larvae (average weight 0.12 gm) @ 100 PL/m³ in each tank. Two diets - one without probiotic (control, T-1) and other with probiotic (test, T-2) were given to the post-larvae of *M. gangeticum* for 90 days. The control feed (T-1) was prepared using ingredients like rice bran, groundnut oilcake, soyabean oilcake, fish meal, prawn meal, starch and vitamin-mineral (Table 1). They were mixed together with vegetable oil and adequate quantity of water to get homogenous dough, pelletized (2.4 mm dia) and dried. The dietary probiotic "Problend" (GlaxoSmithKline Pharmaceuticals Limited, Mumbai) composed of *Lactobacillus sporogenes* 90,000 million cfu, *Lactobacillus acidophilus* 45,000 million cfu, *Bacillus subtilis* 30,000 million cfu, *Bacillus licheniformis* 30,000 million cfu, *Saccharomyces cerevisiae* 125,000 millon cfu, seaweed extract 100 gm, amylase 24,000 IU, phytase 22,00,000 IU, protease 400,00,000 IU, cellulase 150-250 IU, β-galactosidase 800-1000 IU, lipase 50-100 IU, coated vitamin C 35 gm, thiamin mononitrate 1 gm, vitamin B_6 1 gm, vitamin E, 5,000 IU and sodium benzoate 6 gm (per kg) was procured, premixed with this feed @ 0.8% and cod liver oil was used for binding probiotic in the test feed (T-2). Both the feeds were provided to the post-larvae of *M. gangeticum* daily twice @ 10% biomass. Proximate analysis of feed was done following standard methods (AQAC, 1984). Tanks were cleaned daily and water was exchanged @ 50% twice every week. Average growth of PL was recorded at end of each month taking a minimum sample of 30 specimens. The water quality parameters temperature, pH, dissolved oxygen (DO), total alkalinity, total hardness and dissolved ammonia were monitored at regular intervals by following methods given in APHA (1999). Specific growth rate (SGR) and feed conversion ratio (FCR) of the post-larvae fed on control and experimental diets were calculated (Mishra and Pandey, 2012). These values were evaluated for statistical significance by using ANOVA (SAS, ver. 9.2) and Students "t" tests.

Table 1 : Feed ingredients and proximate composition of the control (T-1) diet.

Ingredients	Percentage	Proximate composition	Percentage
Rice bran	40	Moisture	5.60
Groundnut oilcake	10	Protein	42.00
Soyabean oilcake	10	Fat	4.85
Fish meal	10	Ash	13.70
Prawn meal	18	Carbohydrate	33.92
Starch	10		
Vitamin-mineral mixture	2		

RESULTS AND DISCUSSION

Water quality parameters of tanks were within the optimal range (temperature 24.8-28.3°C, pH 7.6-7.9, DO 4.3-4.5 ppm, total alkalinity 82-87 ppm, ammonia 0.083-0.085 ppm) of larval rearing. Growth of the post-larvae of *M. gangeticum* maintained on the control (T-1) and test (T-2) diets has been summarized in Table 2. Post-larvae kept on the test diet (T-2) recorded significantly ($P < 0.05$) higher growth than those maintained on control diet (T-1) (Table 3). The weight gain percentage in post-larvae on control diet (T-1) was 120% whereas in the test diet (T-2), it registered 251%. Growth increment in prawns given test diet (T-2) was significantly higher ($P < 0.05$) than those fed on control diet (T-2) (Table 3). The specific growth rate (SGR) value in the post-larvae on test diet was 1.39 against 0.88 in control diet. Feed conversion ratio (FCR) reflecting the growth, was 2.35 and 3.60 in the post-larvae kept in the test and control diet, respectively. Survival rate of the prawn in control (T-1) and test diet (T-2) was 65% and 85%, respectively.

Though mode of the action of probioitcs has not yet been explored in details, they benefit the host by - (i) competitive exclusion of pathogenic bacteria, (ii) source of nutrients and enzymatic contribution to digestion, (iii) direct uptake of dissolved organic material mediated by bacteria, (iv) enhancement of the immune response against pathogenic microorganisms and (v) anti-viral effects (Balcazar *et al.*, 2006; Li *et al.*, 2007; Yousefian and Amiri, 2009). Ravi *et al.* (1998) reported the benefits of probiotics in maintaining water quality and enhancing growth rate in *Penaeus indicus*. As shrimp aquaculture production of the world has been decreased by the diseases caused by luminous *Vibrio* and/or viruses, probiotic technology provides a solution to these problems by adding selected bacterial species to displace deleterious bacteria in large aquaculture ponds. Abundance of virulent luminous *Vibrio* strains can be controlled when specially selected probiotic strains of *Bacillus* species are added in ponds.

Rengpiat *et al.* (1998) and Rengpiat (2005) found significantly higher growth of post-larvae of

Table 2 : Growth data of *M. gangeticum* fed on control (T-1) and test (T-2) diet.

Days	Control diet (T-1) weight (gm)	Test diet (T-2) weight (gm)
0 days	0.12 ± 0.029	0.12 ± 0.029
30	0.1986667 ± 0.0176995	0.3593333* ± 0.0216871
60	0.2050000 ± 0.0151904	0.3653333* ± 0.0239383
90	0.1840000 ± 0.0117209	0.3646667* ± 0.0286426

Table 3 : Comparison of growth of *M. gangeticum* on control and test diet.

Source	Sum of Square	Mean Square	F Value	Pr > F
Days	0.00354	0.00177	0.14	0.869
Treat	1.25835	1.25835	99.83	<.0001
Error	2.21836	0.01260		
Corrected	3.48025			

farmed black tiger shrimp (*Penaeus monodon*) with dietary probiotics. During proliferation, *Lactobacillus* produces biologically active lactic acid maintaining intestinal pH in the range of 5.5-7.0 (Vieira *et al.*, 2008; Yousefian and Amiri, 2009). The spore of *Bacillus* species is especially easy to introduce in dry foods and/or *Artemia* (Austin and Allen, 1982; Sugita *et al*, 1998). Improvement in growth of the giant freshwater prawn, *Macrobrachium rosenbergii*, has been recorded by feeding *Bacillus* spp. as probiotic bacteria (Deeseenthum *et al.*, 2007). In the present study, improved feed conversion was observed in post-larvae of *M. gangeticum* maintained on test diet over the control. Better feed utilization efficiency consequent to dietary administration of non-hormonal growth promoter has been reported in common carp by Ahmad and Matty (1989). The probiotic administered might enhance growth by increasing availability of nutrients by change in gut microflora or its absorptive capacity (Moriarty, 1999; Irianto and Austin, 2002; Balcazar *et al.*, 2006; Yousefian and Amiri, 2009; Velmurugan and Rajagopal, 2009). Higher growth is also due to improve feed conversion ratio indicating better utilization of nutrients by the post-larvae of *M. gangeticum*. Lakshmanan and Sundarapandian (2008), Sundarapandian and Babu (2010), Soundarpandian *et al.* (2010), Rajanikant *et al.* (2010) have also observed better growth of *Penaus monodon* in hatchery and grow-out conditions given probiotic treatments. Bogut *et al.* (1998) reported improved feed utilization in aquatic organisms fed probiotics probably indicating enhancement in digestive ability of the epithelium of digestive tract.

Survival of post-larvae of *M. gangeticum* kept on test diet (T-2) was 82% as against 62% in prawns kept on control diet (T-1) which may probably be due to enhancement of the immune response against pathogenic microorganisms (Rengpipat, 2005; Balcazar *et al.*, 2006; Yousefian and Amiri, 2009; Ngo and Fotedar, 2010). Lakshmanan and Sundarapandian (2008), Sundarapandian and Babu (2010), Soundarpandian *et al.* (2010 and Rajanikant *et al.* (2010) have also observed higher survival rate of *Penaus monodon* given probiotic treatment under hatchery and grow-out conditions. It is evident from the present study that the probiotic (Problend) is efficient in promoting higher growth during post-larvae rearing of *M. gangeticum*. The water quality parameters play important role in growth and survival of the post-larvae of *M. gangeticum* which were kept at the optimum levels throughout the culture period (Mishra *et al.*, 2011) and the variations in these parameters were negligible in the test as well as control tanks.

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REFERENCES

Ahmad, T.S. and Matty, A.J. (1989). The effect of feeding antibiotics on growth and body composition of carp, *Cyprinus carpio*. *Aquaculture*, 77: 211-220.

APHA (1985). *Standard Methods for the Examination of Water and Wastewater. 16th Edition*. American Public Health Association, Washington D. C. 1268 p.

AOAC (1984). *Officials Methods of Analysis*. Association of Official Analytical Chemists. Wshington. D.C., USA.

Austin, B and Allen, D.A. (1982). Microbiology of laboratory-hatched brine shrimp (*Artemia*). *Aquaculture*, 26: 369-383.

Balcazar, J.L.; de Blas, I.; Ruiz-Zarzuela, I.; Cunningham, D.; Vendrell, D. and Muzquiz, J.L. (2006). The role of probiotics in aquaculture. *Vetern. Microbiol.*, 114: 173-186.

Bogut, I.; Milakovic, Z.; Bukvic, Z.; Brkic, S. and Zimmer, R (1998). Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). *Czech J. Anim. Sci.*, 43: 231-235.

Chen, S.N.; Huang, S.L. and Kau, G.H. (1992). Studies on the epizootiology and pathogenecity of bacterial infections in cultured giant tiger prawn, *Penaeus monodon* in Taiwan. In: *Proceedings of the Workshop on Disease of Cultured Panaeid Shrimp in Asia and the United States*, Hawaii. p. 195.

Dalmin, G.; Kathiresan, K. and Purushothaman, A. (2001). Effects of probiotic on bacterial population and health status of shrimp in culture pond ecosystem. *Indian J. Exp. Biol.*, 39: 939-942.

Deeseenthum, S.; Leelavatchamas, V. and Brooks, J.D. (2007). Effect of feeding *Bacillus* spp. as probiotic bacteria on growth of freshwater prawn (*Macrobrachium rosenbergii* de Man). *Pakistan J. Biol. Sci.*, 10: 1481-1485.

Denev, S.; Staykov, Y.; Moutafchieva, R. and Beev, G. (2009). Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. *Intl. Aquat. Res.*, 1: 1-29.

Dharmaraj, S. and Kandasamy, D. (2010). Evaluation of *Streptomyces* as a probiotic feed for the growth of ornamental fish, *Xiphophorus helleri*. *Food Technol. Biotechnol.*, 48: 497-504.

Fuller, R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol.*, 66: 365-378.

Habib, A. (2010). *Determination of Survival Rate of Prawn Larvae Using Probiotics*. LAP Lambert Academic Pub. 84 p.

Irianto, A. and Austin, B. (2002). Probiotics in aquaculture. *J. Fish. Dis.*, 25: 633-642.

Jayachandran, K.V. (2001). *Palaemonid Prawns: Biodiversity, Taxonomy, Biology and Management*. Science Publishers, Enfield & Plymouth.

Jayachandran, K.V. and Indira, B (2010). Sustainable exploitation of freshwater prawn diversity of India for food and livelihood security with emphasis on planning. *Indian J. Sci. Res.*, 1: 127-132.

Kanaujia, D.R. (2003). Indian river prawn, *Macrobrachium malcolmsonii* and minor species of commercial importance. In: *Souvenir-International Symposium on Freshwater Prawns 2003* (August 20-23, 2003). College of Fisheries, Kerala Agricultural University, Cochin. pp. 51-58.

Kannaujia, D.R.; Mohanty, A.N.; Mitra, G. and Prasad, S. (2005). Breeding and seed production of Ganga river prawn, *Macrobrachium gangeticum* (Bate) under captive condition. *Asian Fish. Sci.*, 18: 371-389.

Kutty, M.N. (2005). Towards sustainable freshwater prawn aquaculture-lessions from shrimp farming, with special reference to India. *Aquacult. Res.*, 36: 255-263.

Lakshmanan, R. and Soundarapandian, P. (2008). Effect of commercial probiotics on large scale

culture of black tiger shrimp, *Penaeus monodon* (Fabricius). *Res. J. Microbiol.*, 3: 198-205.

Li, P.; Burr, G.S.; Gatlin, D.M. III; Hume, M.E.; Patnaik, S.; Castille, F.L. and Lawrence, A.L.-A.L. (2007). Dietary supplementation of short-chain fructo-oligosaccharides influences gastero-intestinal microbiota composition and immunity characteristics of Pacific white shrimp, *Litopenaeus vannamei*, cultured in a re-circulatory system. *J. Nutr.*, 137: 2763-2768.

Mishra, P. and Pandey, A.K. (2012). Effect of commercial probiotic supplementation on growth and survival of post-larvae of *Macrobrachium gangeticum* (Bate). *J. Exp. Zool. India*, 15: 407-411.

Mishra, P.; Kanaujia, D.R.; Bohidar, K. and Pandey, A.K. (2011). Seed production of Gangetic prawn, *Macrobrachium gangeticum* (Bate), in natural seawater and brine solution. *Natl. J. Life Sci.*, 8: 105-112.

Moriarty, D.J.W. (1997). The role of microorganisms in aquaculture ponds. *Aquaculture*, 151: 333-349.

Moriarty, D.J.W. (1999). Disease control in shrimp aquaculture with probiotic bacteria. In: *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology.* (C.R. Bell, M. Brylinsky and P. Johnson-Green, eds.). Atlantic Canada Society for Microbial Ecology, Halifax. pp. 1-7.

Nair, C.M.; Nambudiri, D.D.; Jose, S.; Sankaran, T.M.; Jayachandran, K.V. and Salin, K.R. (2007). *Freshwater Prawns: Advances in Biology, Aquaculture and Marketing*. Allied Publishers, New Delhi & Mumbai.

New, M.B. (2005). Freshwater prawn farming: global status, recent research and a glance at the future. *Aquacult. Res.*, 36: 210-230.

New, M.B.; Nair, M.C.; Kutty, M.N.; Salin, K.R. and Nandeesha, M.C. (2008). *Macrobrachium: The Culture of Freshwater Prawns*. MacMillan India, Chennai.

Ngo, V.H. and Fotedar, R. (2010). A review of probiotics in shrimp aquaculture. *J. Appl. Aquacult.*, 22: 251-266.

Nimrat, S. and Vuthiphandchai, V. (2011). *In vitro* evaluation of commercial probiotic products used for marine shrimp cultivation in Thailand. *Afr. J. Biotechnol.*, 10: 4643-4650.

Ninawe, A.S. and Selvin, J. (2009). Probiotics in shrimp aquaculture: avenues and challenges. *Crit. Rev. Microbiol.*, 35: 43-66.

Rahman, S.; Khan, S.N.; Naser, M.N. and Karim, M.M. (2009). Application of probiotic bacteria: a novel approach towards ensuring food safety in shrimp aquaculture. *J. Bangladesh Acad. Sci.*, 33: 139-144.

Rajinikanth, T.; Ramaswamy, P. and Ravi, V.S. (2010). Efficacy of probiotics on growth promoters and disinfectants in shrimp grow-out farms. *American-Eurasian J. Agric. Environ. Sci.*, 7: 347-354.

Ravi, V.S.; Ajmalkhan, S. and Rajagopal, S. (1998). Influence of probiotics on growth of Indian white prawn, *Penaeus indicus*. *J. Sci. Ind. Res.*, 57: 752-756.

Rengpipat, S. (2005). Probiotic bacteria - are they beneficial? In: *Diseases in Asian Aquaculture. Vol. V* (P. Walker, R. Lester and M.B. Bonded-Reantaso, eds.). Fish

Health Section, Asian Fisheries Society, Manila. pp. 543-552.

Rengpipat, S.; Rukpratanporn, S.; Piyatiratitivorakul, S. and Menasveta, P. (1998). Probiotics in aquaculture: a case study of probiotics for larvae of the black tiger shrimp (*Penaeus monodon*). In: *Advances in Shrimp Biotechnology* (T.W. Flegel, ed.). National Centre for Genetic Engineering and Biotechnology, Bangkok. pp. 177-181.

Sounderapandian, P. and Babu, R. (2010). Effect of probiotics on the hatchery seed production of black tiger shrimp, *Penaeus monodon* (Fabricius). *Intern. J. Anim. Vet. Adv.*, 2: 9-15.

Sounderapandian, P.; Ramanan, V. and Dinakaran, G.K. (2010). Effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius). *Curr. Res. J. Social Sci.*, 2: 51-57.

Sugita, H.; Hirose, Y.; Matsuo, N. and Deguchi, Y. (1998). Production of the antibacterial substances by *Bacillus* sp. Strain NM 12 an intestinal bacterium of Japanese costal fish. *Aquaculture*, 165: 269-280.

Velmurugan, S. and Rajagopal, S. (2009). Bacterial uses of probiotics in mass scale production of marine ornamental fish. *Afr. J. Microbiol. Res.*, 3: 185-190.

Vieira, F.N.; Neto, C.C.B.; Mourino, J.L.P.; Jatoba, A.; Ramirez, C.; Martins, M.L.; Barracco, M.A.A.M. and Vinatea, L.A. (2008). Time-related action of *Lactobacillus plantarum* in the bacterial microbiota of shrimp digestive tract and its action as immunostimulant. *Pesq. Agropec. Bras. (Brasília)*, 43: 763-769.

Vijayakumaran, M. (2001). Probiotics in aquaculture. In: *Perspectives in Mariculture* (N.G. Menon and P.P. Pillai, eds.). Marine Biological Association of India (CMFRI), Cochin. pp. 369-380.

Yousefian, M. and Amiri, M.S. (2009). A review of the use of prebiotic in aquaculture for fish and shrimp. *Afr. J. Biotechnol.*, 8: 7313-7318.

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HEROIN ABUSE AFFECTS MALE REPRODUCTIVE FUNCTION IN ALBINO *MUS NORVEGICUS*

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ABSTRACT

Sublethal heroin administration (0.50 LD_{50}; 13.5 mg/kg/day) in albino *Mus norvegicus* elicited significant ($P < 0.001$) decline in serum LH levels on day 1 (9.66±0.81 ng/ml) and 4 (9.54±0.45 ng/ml) with the minimal value on day 30 (7.84±0.42 ng/ml). Serum testosterone level of heroin treated rats also depicted a progressive decline ($P < 0.05$) on day 1 (1.48±0.08 µg/100 ml) and 4 (1.40±0.05 µg/100 ml) while minimum value ($P < 0.001$) was recorded on day 30 (0.81±0.13 µg/100 ml). Seminiferous tubules of heroin treated rats showed massive degenerative changes in the spermatogonial cells. Number of Sertoli cells was also reduced but spermatocytes as well as spermatids were seen attached to it. The lumina showed debris of spermatogenic cells with scanty spermatozoa. Leydig cells located in the intertubular space also showed atrophy and vacuolization.

Key words: Heroin, serum LH, serum testosterone, Leydig cells, Mus norvegicus.

Heroin (Brown Sugar) abuse is a burning problem of the society. Chronic abuse of heroin has diverse effects on various body systems due to widespread distribution of specific receptors in many tissues and organs (Siegel *et al.*, 1982). The drug (diacetylmorphine) is metabolized into 6-acetylmorphine and subsequently to morphine in the human body (Martin, 1984; Sawynok, 1986; Cami and Farre, 2003). Despite of long history of its clinical therapeutic use and protracted abuse by addicted subjects, little is known regarding possible influences of the drug on the endocrine system (George *et al.*, 2005; Brown *et al.*, 2006; Al-Gommer *et al.*, 2007; Bhoir *et al.*, 2007, 2009: Barai *et al.*, 2009a, b). There are clinical evidences suggesting inhibition of some parameters of sexual function in human addicted to heroin, most notably impaired libido, impotency and delayed ejaculation (McKendry *et al.*, 1983; Weiland and Yunger, 1985. Addiction is usually considered as psychological problem directly related to narcotics use. Plasma level of luteinizing hormones (LH) was normal in both methadone maintained as well as active heroin addicts. Chronic narcotic administration produced marked atrophy of the secondary sex organs and suppression of plasma testosterone level in male rat. About 87% reduction in serum testosterone level was associated with the atrophy of seminal vesicle, prostrate and epididymis (Cicero *et al.*, 1975, 1976). The long-term methadone use impairs the function of secondary sexual organs in human beings too. There exist reports demonstrating lower testosterone level in heroin and methadone users. An attempt has, therefore, been made to evaluate the effect of sublethal (0.50 LD_{50}; 13.5 mg/kg/day) heroin administration on serum LH, testosterone and testicular morphology of albino *Mus norvegicus*

MATERIALS AND METHODS

Healthy male *Mus norvegicus* (Wistar strain) weighing 150-200 gm were procured from Hoffkin Institute, Parel, Mumbai and housed in specially made

plastic cages. They were acclimatized under the ambient laboratory conditions (temperature 28±2°C; photoperiod 14L:10 D) for 10 days, fed *ad libitum* on rat feed (Lipton, Hindustan Lever Ltd., Bangalore) and clean water was provided for drinking. Care was taken to ensure that the rats were treated in the most humane and ethically accepted manner. 60 male rats were randomly selected and divided into two equal groups - experimental and control. Heroin (85% pure) was dissolved initially in small quantity of alcohol and diluted with physiological saline to prepare the test dose of 0.50 LD_{50} (13.5 mg/kg/day). The drug was administered through subcutaneous (s.c.) route to the experimental rats while the control rats received equal volume (0.2 ml/kg body weight) of the physiological saline. Blood samples were collected on day 1, 4 and 30 and circulating levels of LH and testosterone were measured by radioimmunoassay (RIA) techniques at Tata Cancer Research Centre, Bhabha Atomic Research Centre (BARC), Mumbai.

The animals were killed on day 30 and their testes were surgically removed, washed in normal saline and fixed immediately in Bouin's fluid for light microscopy. After 24 hours, the tissues were washed throughly in water dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. The sections were cut at 5 µm and stained in hematoxylin-eosin (H&E) and buffered toluidine blue for light microscopic studies. For electron microscopy, the tissues were removed immediately after the sacrifice and sliced into 1 mm pieces to allow better penetration of fixative chemical (3% ice-cold glutaraldehyde) for 12 hours followed by 4 hours in 0.1 M cacodylate buffer. They were rinsed in buffer and post-osmicated in 1% osmium tetroxide (OsO_4) for 1-2 hours and dehydrated in ascending alcohol grades followed by propylene oxide and embedded in resin polymerized at 60°C. The blocks were prepared in araldite. 1 µm thin sections were cut with glass knife on an LKB-2000 ultramicrotome. Sections were mounted on glass slides and stained with buffered toluidine blue. Ultrathin sections of the selected blocks were cut with glass knife, picked up on copper grids and stained with uranyl acetate and lead citrate for final observation under ZEIM-EM-109 electron microscope.

RESULTS AND DISCUSSION

Serum LH level of control rat varied between 26.86±5.06 and 28.26±7.19 ng/ml while testosterone from 2.16±0.58-2.30±0.32 µg/100 ml during the experimental period. Sublethal heroin administration induced significant ($P < 0.001$) decline in serum LH level on day 1 (9.66±0.81 ng/ml) and 4 (9.54±0.45 ng/ml) with the minimal value on day 30 (7.84±0.42 ng/ml). Serum testosterone level of heroin treated rats also depicted a progressive decline ($P < 0.05$) on day 1 (1.48±0.08 µg/100 ml) and 4 (1.40±0.05 µg/100 ml) while minimum value ($P < 0.001$) was recorded on day 30 (0.81±0.13 µg/100 ml).

Testis of control rat exhibited convoluted seminiferous tubules, the wall of which was consisted of basement membrane and a lining of stratified epithelium. The epithelium consisted of Sertoli (or supporting) cells and the spermatogenic cells. Sertoli cells were tall, irregularly columnar and extended from basal lamina to the lumen. The spermatogenic cells exhibited uniform cellular arrangement with five maturation stages - spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 1). The early spermatids were seen with acrosome formation. The mitochondria were dispersed throughout the cytoplasm. Sertoli cells were also seen with normal architecture. Leydig cells were seen in the interlobular area of the seminiferous tubules. The spermatogenic cells depicted normal architecture (Fig. 2).

Seminiferous tubules of heroin treated rats showed massive degenerative changes in the spermatogonial cells. Though the spermatogonia resting on the basement membrane, were in active phase of division but number of these cells was reduced. Primary spermatocytes, secondary spermatocytes and spermatids also exhibited degenerating changes and the intercellular space between these cells was increased (Fig. 3). Number of Sertoli cells was also reduced but spermatocytes as well as spermatids were seen attached to it. The

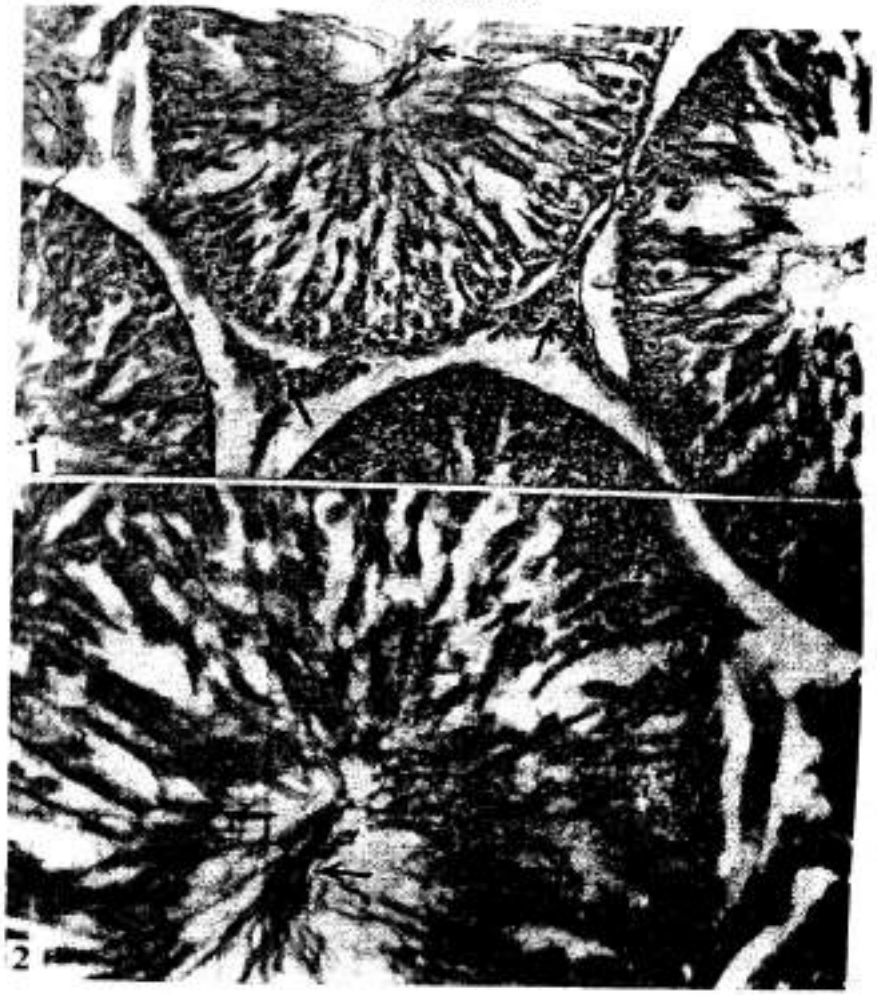

Fig. 1: Seminiferous tubules of control *Mus norvegicus* exhibiting different stages of spermatogenesis with spermatozoa in the lumen (arrow) and Leydig cells in the intertubular spaces (broken arrow). H&E. x 250.

Fig. 2: Magnified view depicting germinal epithelium and different stages of spermatogenesis. Mark spermatozoa in the lumen (arrow). H&E. x 400.

Fig. 3: Testis of heroin treated rat on day 30 showing degenerative changes in seminiferous tubules. Mark the lumen with cellular debris and scanty spermatozoa. H&E. x 250.

Fig. 4: Seminiferous tubules of heroin treated rat on day 30 depecting massive degeneration of spermatogenic cells. Mark the atrophied Leydig cells with vacuolated cytoplasm (arrow). H&E. x 400.

lumina showed debris of spermatogenic cells with scanty spermatozoa (Fig. 4). Leydig cells located in the intertubular space also showed atrophy and vacuolization.

Ultrastructural observations of the testis of heroin treated rat showed Leydig cells in angular interstices between the seminiferous tubules with indented nucleus. The cytoplasm showed hypertrophied mitochondria, Golgi bodies with dilated cisternae, multivesicular bodies and membrane limited lysosomes. The myoid layer of lamina propria was clearly seen and Sertoli cells were with infolded nuclear membrane. Spermatogonia, resting firmly on lamina propria, were separated by continuous tight junctional complexes. Cytoplasm of Sertoli cells showed numerous elongated mitochondria, lipid droplets of varying sizes and density, endoplasmic network and scattered multi-vesicular bodies. Onset of vacuolization was prominent at some places. In the lumina, spermatids were clearly visible with the formation of acrosomal granule, acrosomal cap and a well-established acrosomal membrane. Extreme cytoplasmic degeneration in lumina of the seminiferous tubule showed lysosomal activity, prelysosomal vesicular structures and many hypertrophied mitochondria on day 30 of the heroin administration.

The effects of narcotics on hormonal and sexual physiology are not well understood (Brambilla *et al.*, 1977; Wang *et al.*, 1978; Malik *et al.*, 1992; Daniell, 2002; George *et al.*, 2005). It was found that long-term methadone administration in human males markedly impaired the function of secondary sex organs and depressed testosterone level (Cicero *et al.*, 1975, 1976). Methadone induced reduction in serum testosterone level has been recorded in human (Azizi *et al.*, 1973; Mendelson *et al.*, 1975a, b). Martin *et al.* (1973) observed that methadone decreases gonadotropin level while Cushman (1973) found reduction in luteinizing hormone (LH) and testosterone level in male heroin or methadone users. Chronic cocaine abuse is associated with significant decrease in libido and reproductive function (Washton *et al.*, 1984). Impotence and gynecomastia have been observed in male cocaine users while major derangement in menstrual cycle function has been recorded in case of women leading to amenorrhea and infertility (Siegel *et al.*, 1982; Cocores *et al.*, 1985). Though cocaine administration did not induce significant change in LH and testosterone levels in man and rhesus monkey ((Mendelson *et al.*, 1989; Mello *et al.*, 1993), there exists the possibility that opioids may effect on the gonadal portion of the hypothalamo-pituitary-gonadal (HPG) axis (Brambilla *et al.*, 1979; Adam *et al.*, 1993). Brambilla *et al.* (1977, 1979), Wang *et al.* (1978), Bolelli *et al.* (1979), Mendelson *et al.* (1980) and Malik *et al.* (1992) observed suppression in the levels of LH, FSH (follicle stimulating hormone) and testosterone levels in the human subjects addicted to heroin. Though there are indications of the involvement of hypothalamus and higher centres of brain in heroin-induced alterations of reproductive physiology (Brambilla *et al.*, 1979), the observed decline in LH and testosterone (T) levels concomitant with atrophy and vacuolization in Leydig cells as well as degenerative changes in seminiferous tubules of *Mus norvegicus* suggest that heroin induced changes is mediated through hypophysial-gonadal axis in Wistar rat.

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REFERENCES

Adams, M.L.; Sewing, B.; Forman, J.B.; Meyer, E.R. and Cicero, T.J. (1993). Opioid induced

suppression of rat testicular function. *J. Pharmacol. Exp. Ther.*, **266**: 323-328.

Al-Gommer, O.; George, S. and Haque, S. (2007). Sexual dysfunctions in male opiate users: a comparative study of heroin, methadone and buprenorphine. *Addict. Disord. Their Treat.*, **6**: 137-143.

Azizi, F.; Vagenakis, A.G.; Longcope, C.; Ingbar, S.H. and Braverman, L.E. ((1973). Decreased serum testosterone concentration in male heroin addicts. *Steroid*, **23**: 467-472.

Barai, S.R.; Suryawanshi, S.A. and Pandey, A.K. (2009a). Levels of plasma sodium and potassium levels as well as alterations in adrenal cortex of *Rattus norvegicus* to sublethal heroin administration. *J. Environ. Biol.*, **30**: 253-258.

Barai, S.R.; Suryawanshi, S.A. and Pandey, A.K. (2009b). Responses of plasma calcium and inorganic phosphate levels, parathyroid gland and calcitonin-producing C cells of *Rattus norvegicus* to sublethal heroin administration. *J. Environ. Biol.*, **30**: 917-922.

Bhoir, K.K.; Suryawanshi, S.A. and Pandey, A.K. (2007). Responses of serum adrenocorticotropic hormone (ACTH) and cortisol levels as well as adrenal cortex of *Rattus norvegicus* to sublethal heroin administration. *J. Ecophysiol. Occup. Hlth.*, **7**: 185-191.

Bhoir, K.K.; Suryawanshi, S.A. and Pandey, A.K. (2009). Effects of sublethal heroin administration on serum thyroid stimulating hormone (TSH), thyroid hormones (T_3, T_4) and thyroid gland of *Rattus norvegicus*. *J. Environ. Biol.*, **30**: 989-994.

Bolelli, G; Lafisca, S.; Flamigni, C.; Lodi, S.; Franceschetti, F.; Filicori, M. and Mosca, R. (1979). Heroin addiction: relationship between the plasma levels of testosterone, dihydrotestosterone, androstenedione, LH, FSH and the plasma concentration of heroin. *Toxicology*, **15**: 19-29.

Brambilla, F.; Sacchetti, E. and Brunetta, M. (1977). Pituitary-gonad function in heroin addicts. *Neuropsychobiology*, **3**: 160-166.

Brambilla, F.; Resele, L.; de Maio, D. and Nobile, P. (1979). Gonadotropin response to synthetic gonadotropin hormone-releasing hormone (GnRH) in heroin addicts. *Am. J. Psychiat.*, **136**: 314-317.

Brown, T.T.; Wisniewski, A.B. and Dobs, A.S. (2006). Gonadal and adrenal abnormalities in drug users: cause of consequence of drug use behaviour and poor health. *Am. J. Infect. Dis.*, **2**: 130-135.

Cami. J. and Farre, M. (2003). Drug addiction. *N. Eng. J. Med.*, **349**: 975-986.

Cicero, T.J.; Bell, R.D.; Wiest, W.G.; Allison, J.H. Polakoski, K. and Robins, E. (1975). Function of the male sex organs in heroin and methadone users. *N. Engl. J. Med.*, **292**: 882-887.

Cicero, T.J.; Mayer, E.R.; Bell, R.D. and Koch, G.A. (1976). Effect of morphine and methadone on serum testosterone and luteinizing hormone levels and on the secondary sex organs of the male rat. *Endocrinology*, **98**: 367-372.

Cocores, J.A.; Dackis, C.A. and Gold, M.S. (1985). Sexual dysfunction: secondary to cocaine abuse in two patients. *J. Clin. Psychiat.*, **47**: 384-385.

Cushman, P. Jr (1973). Plasma testosterone in narcotics addiction. *Am. J. Med.*, **55**: 452-458.

Daniell, H. (2002). Hypogonadism in men consuming sustained-active oral opioids. *J. Pain*, **3**: 377-384.

George, S.; Murali, V. and Pullickal, R. (2005). Review of neuroendocrine correlates of chronic opiate misuse: dysfunctions and pathophysiological mechanisms. *Addict. Disord. Their Treat.*, **4**: 99-109.

Malik, S.A.; Khan, C.; Jabbar, A. and Iqbal, A. (1992). Heroin addiction and sex hormones in males. *J. Pak Med. Assoc.*, **42**: 210-212.

Martin, W.R. (1984). Pharmacology of opioids. *Pharmacol. Rev.*, **35**: 282-323.

Martin, W.R.; Jesinski, D.R.; Haertzen C.A.; Kay, D.C.; Jones, B.E.; Mansky, P.A. and Carpenter, R.W. (1973). Methadone: a re-evaluation. *Arch. Gen. Psychiat.*, **28**: 286-295.

McKendry, J.B.; Collins, W.E.; Silverman, M.; Krul, L.E.; Collins, J.P. and Irvine, A.H. (1983). Erectile impotence: a clinical challenge. *Can. Med. Assoc. J.*, **128**: 653-663.

Mello, N.K.; Lukas, S.E.; Mendelson, J.E and Drieze, J. (1993). Naltrexone-buprenorphine interactions: effects on cocaine self-administration. *Neuropsychopharmacology*, **9**: 21-224.

Mendelson, J.H.; Mendelson, J.E. and Patch, V.D. (1975a). Plasma testosterone levels in heroin addiction and during methadone maintenance. *J. Pharmacol. Exp. Ther.*, **192**: 211-217.

Mendelson, J.H.; Meyer, R.E.; Ellingboe, J.; Mirin, S.M. and DeDaugle, M. (1975b). Effect of heroin and methadone on plasma cortisol and testosterone. *J. Pharmacol. Exp. Ther.*, **195**: 296-302.

Mendelson, J.H.; Ellingboe, J.; Kuehnle, J.C. and Mello, N.K. (1980). Heroin and naltrexone effects on pituitary-gonadal hormones in man: interaction of steroid feedback effects, tolerance and supersensitivity. *J. Pharmacol. Exp. Ther.*, **214**: 503-506.

Mendelson, J.H.; Mello, N.K.; Toeh, S.K.; Ellingboe, J. and Cochin, J. (1989). Cocaine effects on pulsatile secretion of anterior pituitary, gonadal and adrenal hormones. *J. Clin. Endocrinol. Metab.*, **69**: 1256-1260.

Rasheed, A and Tareen, I.A.K. (1995). Effects of heroin on thyroid function, cortisol and testosterone levels in addicts. *Polish J. Pharmacol.*, **47**: 441-447.

Sawynok, J. (1986). The therapeutic use of heroin: a review of the pharmacological literature. *Can. J. Physiol. Pharmacol.*, **64**: 1-6.

Siegel, P.; Hinson, R.E.; Krank, H.D. and McCully, J. (1982). Heroin "overdose" deaths: contribution of drug-associated environmental cues. *Science*, **21**: 436-437.

Sporer, K.A. (1999). Acute heroin overdose. *Ann. Intern. Med.*, **130**: 584-590.

Wang, C.; Chan, V. and Yeung, R.T. (1978). The effect of heroin addiction on pituitary-testicular function. *Clin. Endocrinol.*, **9**: 455-461.

Wieland, W.F. and Yunger, M.M. (1985). Sexual effects and side effects of heroin and methadone. In: *Proceedings of the Third International Conference on Methadone Treatment*. National Institute of Mental Health. PHS Pub. No. 2172. 50p.

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EFFECT OF VITAMIN E SUPPLEMENTATION ON ANIMAL PERFORMANCE OF ARSENIC FED GROWING KIDS

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ABSTRACT

Experiment was conducted on Twelve crossbred goat kids (Alpine x Beetle) were divided into three groups with four animals in each group. The nutrient requirement of the animal was met by feeding concentrate mixture, berseem fodder and wheat straw as per NRC (1981). One group served as control whereas animals in group T-1 were fed 50 ppm As and group T-2 was provided 50 ppm As along with vitamin E @ 50 IU/kg of DM. The DM intake of the animals was recorded every day and body weight was taken weekly. Results showed that daily DMI and body weight gain were not adversely affected by the administration of As (P>0.05). Initial body weight (kg) of the animals was 10.50, 10.12 and 10.25 in C, T-1 and T-2, groups respectively and at 90 days the body weight in these respective groups was 14.75, 14.00 and 14.25 kg showing that there was not any adverse effect of As administration (P>0.05). The DMI per 100 kg body weight was 3.42, 3.43 and 3.39 in C, T-1 and T-2 groups respectively again showing insignificant effect (p>0.05) due to dietary treatments.

Keywords: Kids, performance, arsenic and vitamin E

The livestock population is exposed to a wide range of toxicants from various sources (Radostits *et al.*, 1994). Amongst the toxicants, heavy metals like arsenic, lead, cadmium, mercury etc. are wide spread and dangerous to animal health. With rapid industrialization, urbanization and unprecedentedly increase in population, pollutants such as heavy metals from automobiles and other sources are continuously increasing in the environment. Besides this, ground water also poses a major threat of exposing animals and human beings to toxic levels of heavy metals in some geographical areas in the world. Arsenic causes decrease in growth rate in monogastric animals (Glattre *et al.*, 1995), while in farm animals its implication is meagerly reported. Forsberg (1978) reported that the arsenic concentration (5.7 ppm) which is nontoxic to sheep may be inhibitory to rumen micro flora. Therefore, if the animal is exposed to low concentration of arsenic for a longer time, the microflora will be destroyed and animal will suffer from decreased productivity and ill health. Toxicity of arsenic varies with factors such as oxidation state, solubility, and species of animal involved and duration of exposure.

MATERIALS AND METHODS

During growth trial of 13 weeks duration, the goat kids were provided concentrate mixture, berseem fodder and wheat straw. The concentrate mixture provided to animals contained maize grain, ground nut cake (Expeller), mustard oil cake, wheat bran, deoiled rice bran, mineral mixture and common salt. The chemical composition of all the dietary feed stuffs is given. The CP content of concentrate mixture was 14.76 % and in wheat straw and berseem it was 3.83 and 15.86 % respectively. The NDF content of concentrate mixture, wheat straw and berseem was

21.07, 82.19 and 44.87 % respectively. The acid detergent lignin of concentrate mixture, wheat straw and berseem was estimated to be 1.11, 9.50, and 6.90 % respectively. These values were in close agreement with values reported by Dey *et al.* (2004) and Thakur and Sharma (1999). The kids were given weighed quantity of concentrate mixture, wheat straw and berseem fodder to meet their nutrient requirement as per NRC (1981). To kids of T-1, 50 ppm of arsenic was given daily whereas to kids of T-2, 50 IU/kg DM of vitamin E was also supplemented along with 50 ppm of arsenic. Calculated quantities of As and vitamin E were weighed in a capsule and given orally to animals everyday in the morning. Data obtained on various parameters were tabulated and statistically analyzed using analysis of variance (ANOVA) technique as per Snedecar & Cocharan (1994) in RBD

RESULTS AND DISCUSSION

An average dry matter intake, animal performance in term of weight gain per day, feed conversion ratio (calculated as DMI/kg gain) or percent feed efficiency (calculated as the gain in body weight per unit of feed intake) of goat kids fed on different dietary treatments i.e. control, T-1 and T-2 for 90 days of experimental period is presented in table. It was obsepved that the gain (g) during a period of 13 weeks in control, T-1 and T-2 groups was 47.22±6.00, 43.11±6.15 and 44.44±6.00. It was observed that though there was a decrease in weight gain in T-1 group as compare to control and in T-2 group the weight gain was more as compared to T-1 group which indicated that vitamin E supplementation exhibited a slight protective effect but statistically these results were not significant (P>0.05). The feed conversion ratio in control, T-1 and T-2 groups was

Table 1 : Animal performance, feed intake and percent feed efficiency in arsenic fed goat kids by supplementation of vitamin E

Particulars	Groups		
	Control	T- 1	T- 2
Av. initial body wt.(kg)	10.50±1.13	10.12 ±0.74	10.25±1.05
Av. final body wt. (kg)	14.75±0.95	14.00±0.71	14.25±0.51
Total body wt. gain (kg)	4.25±0.74	3.88±0.54	4.00±0.66
Gain /day (g)	47.22±6.00	43.11±6.15	44.44±6.00
Av. DMI /day (g)	420.00±14.61	416.08±14.08	420.43±15.22
From concentrate(g)	189.00±3.26	187.23±4.08	189.19±4.13
From berseem(g)	105.00±1.76	104.02±2.15	105.11±1.32
From wheat straw(g)	126.00±4.60	124.82±4.89	126.13±5.61
DMI (% body wt.)	3.42±0.02	3.43±0.03	3.39±0.03
Feed conversion ratio (kg DM / kg gain)	8.89±0.86	9.65±1.70	9.46±1.46
% Feed efficiency (kg gain/ 100 kg DM)	11.24±1.46	10.36±1.51	10.57±1.35

calculated to be 8.89±0.86, 9.65±1.70 and 9.46±1.46 g respectively, whereas the percent feed efficiency in these groups was 11.24±1.46, 10.36±1.51 and 10.57±1.35 respectively. All the parameters viz. body weight gain per day, feed conversion ratio and feed efficiency did not show any significant effect due to supplementation of 50 ppm of As or 50 IU/kg DM of vitamin E. It was due to the reason that As supplementation level was within the maximum permissible level.

Another reason might be due to the fact that As is cumulative in nature therefore, to exhibit its adverse effect a longer duration is required and the present study was of 90 days only, which appears to be insufficient to draw some conclusion

CONCLUSION

It was concluded that there was a non-significant effect of Arsenic addition @ 50 ppm for 90 days had no adverse effect on DMI and body weight gain and FCR in goat kids.

REFERENCES

Day, A.; Sehal, J.P.; Puniya, A.K. and Singh, K. 2004. Influence of an anaerobic fungal culture administration on growth rate, Ruminal fermentation and nutrient digestibility in calves. *Asian-Australian J. Anim. Sci.*, 17(6): 820-824.

Frosberg, C.W.1978. Some effects of arsenic on the rumen fermentation microflora; an in vitro study. *Can.J.Microbiol.* 24: 36-44.

Glattre, E.; Mravacova, A.; Lener, J.; Vobecky, M.; Egertova, E. and Mysliveckova, M. 1995. Study of distribution and interaction of arsenic and selenium in rat thyroid. Biological Trace Element Research,49(2-3):177-186.

Thakur, S.S. and Sharma, D.D. 1999. Bacterial and volatile fatty acid production rates in crossbred calves fed chemically preserved maize. *Indian J. Anim. Nutr.* 16:24-29.

NRC, 1981. Nutrient requirement of goats. Angora, Dairy and Meat goats in Temperate and Tropical countries, series 15. National Acad. Sci. Washington, D.C. pp 10-12 (Chapter 83).

Radostits, O.M.; Blood, D.C. and Gay, C.C.1994. Veterinary Medicine 8th Edition, Bailliere Tindall, London,pp.1480-1484.

Snedecor, C.W. and Cochran, W.G. 1994. Statistical method. Iowa State University press, Ames, Iowa.

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EFFECT OF METABOLIC SIZE OF COWS ON CHEMICAL QUALITY OF THEIR RAW MILK

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ABSTRACT

The study was undertaken on Effect of metabolic size of cows on chemical quality of their raw milk. The crossbred milch herd of the Animal Husbandry Department of the SHIATS, Allahabad was subjected to Californian Mastitis Test and 12 cows with negative CMT were selected. All experimental animals managed under similar management conditions. Cows were milked by dry full hand method of milking. Two streams of fore milk from each quarter of udder were discarded. Milk samples were directly from the udder and analyzed for determination of total solid (TS), fat, solid not fat (SNF), lactose, protein, ash, water, sp. gr. and acidity percentage in milk. The results showed that metabolic size of cows had no significant effect on sp. gr. and protein in milk of cows however it had a significant effect on fat, lactose, S.N.F., T.S. and water percentage in milk. It was revealed that milk of better chemical quality containing significantly more fat and total solid was produced by cows of 298 to333 metabolic sizes followed by cows of 226 to 261 metabolic size, 262 to 297 metabolic size and cows of 190 to 225 metabolic sizes.

***Keywords** : Metabolic size, crossbred cows, chemical parameters.*

India is an agriculture country basically a rural oriented and land based with 76.27% of rural population, 1/5 of the world's population of cattle and more than ½ of world's buffaloes. The cows and bullocks are the backbone of the agriculture and play a major role in the rural economy. Milk occupies a unique position among foods, being complete food for infants, good supplementary food for people of all ages and essential protective food for sick and invalids. Milk proteins are highly nutritious that effectively supplements poor quality vegetable proteins in a mixed diet. Milk is a rich source of all vitamins especially vitamin A, riboflavin and vitamin B_{12}. Milk is a rich source of calcium in the best available. There is great deal of variation in the composition of milk, even with the same animal it is not always the same. Among the constituents the fat content of the milk is most variable. The other constituents vary in the order- Protein, Lactose and Ash. The factors responsible for such variations in the composition are species of animal, breed, stage of lactation individually, variation from milking to milking length of interval between milking, first and last milk, type of feed, physical condition of the animal, environment, disturbance at milking time etc. Whether these milk ingredients are influenced by the metabolic size of cow is not yet ascertained.

MATERIALS AND METHODS

The experiment was conducted during 2011-2012 in Department of Animal Husbandry and Dairying, SHIATS, Allahabad. Metabolic size of cows of SHIATS dairy farm was determined by standard formula Metabolic size = Body weight x 0.75 because size of an animal is proportional to its metabolic rate (Prasad and Neeraj, 2009). Only 12 healthy cows free from mastitis and other noticeable injuries were selected and divided into 4 groups of three cows in each for four treatments of metabolic size viz. 190 to

225 (M_1), 226 to 261 (M_2), 262 to 296 (M_3), 297 to 333 (M_4). These samples were used for chemical quality to determine total solid (TS), Fat, Solid not fat (SNF), lactose, protein, ash, water, sp. gr. and acidity percentage in milk. Crossbred milch herd of the SHIATS dairy farm was subjected to Californian Mastitis Test (CMT) and cows with negative CMT were selected for the experiment. All cows were housed in a tail to tail barn and managed under similar management conditions. 200 ml milk sample from each cow was collected directly into sterilized conical flask of 250 ml. capacity and plugs replaced immediately. The samples were used for chemical quality to determining total solid (TS), Fat, Solid not fat (SNF), lactose, protein, ash, water, sp. gr. and acidity percentage in milk. Milk samples were analyzed to determine chemical parameters as per method of AOAC (1995). Total solids (TS) percent, Fat percent, Solid not fat (SNF) percent, Protein percent, Lactose percent, Ash percent, Acidity percent, Specific gravity (sp. gr.), and Water percent. The Lactometer was used for rapid determination of specific gravity of Murthy (1993) analyzed to determine influence of metabolic size on different chemical parameters of raw milk. The data on compositional ingredients were tabulated and subjected to analysis of variance techniques (ANOVA) as per randomized block design (RBD) of Snedecar and Cochran (1994) to determine influence of metabolic size on different chemical parameters of raw milk.

RESULTS AND DISCUSSION

The mean fat percent was recorded in milk of cows of M_3 metabolic size (5.8) followed by milk of cows of metabolic size M_4 (4.55), M_1 (4.10) and M_2 (3.97). The differences in fat percent due to metabolic size were significant. The mean protein percent was recorded in milk of cows of M_1 metabolic size (3.64) followed by milk of cows of metabolic size M_2 (3.57), M_3 (3.52) and M_4 (3.31). The differences in protein percent due to metabolic size were not significant.

The mean lactose percent was recorded in milk of cows of M_3 metabolic size (4.76) followed by milk of cows of metabolic size M_2 (4.37), M_1 (4.32) and M_4 (4.17). The differences in lactose percent due to metabolic size were significant. The mean ash percent was recorded in milk of cows of M_1 metabolic size (0.69) followed by milk of cows of metabolic size M_2 (0.68), M_4 (0.67) and M_3 (0.67). The differences in ash percent due to metabolic size were significant. The mean total solid percent was recorded in milk of cows of M_4 metabolic size (14.05) followed by milk of cows of metabolic size M_1 (13.63), M_3 (13.53) and M_2 (13.47). The differences in total solid percent due to metabolic size were significant. The mean S.N.F. percent was recorded in milk of cows of M_2 metabolic size (8.78) followed by milk of cows of metabolic size M_4 (8.77), M_3 (8.53) and M_1 (8.02). The differences in S.N.F. percent due to metabolic size were significant. The mean acidity percent was recorded in milk of cows of M_4 metabolic size (0.14) followed by milk of cows of metabolic size M_3 (0.13), M_1 (0.13) and M_2 (0.13). The differences in acidity percent due to metabolic size were significant. The mean specific gravity percent was recorded in milk of cows of M_4 metabolic size (1.02) followed by milk of cows of metabolic size M_3 (1.02), M_2 (1.02) and M_1 (1.02). The differences in specific gravity percent due to metabolic size were not significant. The mean water percent was recorded in milk of cows of M_2 metabolic size (86.55) followed by milk of cows of metabolic size M_3 (86.47), M_1 (86.33) and M_4 (85.77). The differences in water percent due to metabolic size were significant.

CONCLUSION

The effect of metabolic size of cows on chemical quality of raw milk was conducted. Sp. gr. remained unaffected by metabolic size of cows, but metabolic size of cows had no effect on protein in milk of cows however it had a significant effect on fat, lactose, S.N.F., T.S. and water percentage in milk. It was revealed that cows of 298 to 333 metabolic size produced milk of better chemical quality containing

Table 1 : Mean values of different parameters according to metabolic size (M) of cows.

Parameter	Mean values of parameters			
	M_1 (190-225)	M_2 (226-261)	M_3 (262-297)	M_4 (298-333)
Fat percent	4.10[a]	5.80[b]	3.97[a]	4.55[c]
Protein percent	3.64[a]	3.52[a]	3.57[a]	3.31[a]
Lactose percent	4.32[a]	4.37[ab]	4.76[b]	4.17[a]
Ash percent	0.69[b]	0.67[a]	0.68[ab]	0.67[a]
T.S. percent	13.63[a]	13.47[a]	13.53[a]	14.05[b]
S.N.F. percent	8.02[b]	8.78[c]	8.53[c]	8.77[c]
Water percent	86.33[a]	86.55[a]	86.47[a]	85.77[b]
Acidity percent	0.13[a]	0.13[a]	0.13[a]	0.14[a]
Specific gravity	1.02[a]	1.02[a]	1.02[a]	1.02[a]

significantly more fat and total solid followed by cows of 226 to 261 metabolic size, 262 to 297 metabolic size and cows of 190 to 225 metabolic sizes.

REFRENCES

AOAC, 1995. Assoc of official analytical chemists, 16th Ed. P.O. Box 540 Benjamin Franklin Station Washington D.C. 20, 0444.

Murthy, M.K.R. 1993. Specific Gravity of milk and its uses in Dairy Industry. *Indian Dairyman*, 6:241-246.

Prasad, J. and Neeraj, 2009 Animal husbandry and Dairy Science, Kalyani Publications, P-3.

Snedecor, G.W. and Cochran, W.G. 1994 Statistical method 8th Ed. The IOWA State University, Press Amer IOWA U.S.A.

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EFFECT OF Cd × Zn INTERACTION ON THE UPTAKE OF Cd BY MAKOY

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ABSTRACT

A pot experiment was arranged to study the effect of Cd × Zn interaction on the uptake of Cd by makoy (*Solanum nigrum* L.). Cadmium was applied as $CdCO_3$ at the rate of 0, 5, 10 and 15 mg kg^{-1} and Zn was applied as $ZnSO_4 7H_2O$ at the rate 0, 20, 40 and 60 mg kg^{-1}. It was observed that the yield of *Solanum nigrum* L. was decreased with the single application of cadmium. But when it was applied with zinc, the yield was increased. The reduced uptake of Cd was observed in Zn treated pots. The response of Zn was observed ameliorative in Cd contaminated pots.

Key words: *Cadmium, zinc, solanum nigrum L, uptake*

Heavy metal pollution of soils has dramatically increased in recent decades due to the discharge of waste and wastewater from anthropogenic sources (Ghosh and Singh, 2005). This situation has become a critical environmental issue owing to the potential adverse ecological effects of the pollutants (An, 2004). Among the heavy metals, cadmium (Cd) has been considered to be one of the most serious metal contaminants since the Itai-Itai disease reported in Japan. As a non-essential element for living organisms, Cd has a very high mobility in soil-plant systems, with propensity to adversely effect both human health and the functioning of ecosystems (Perronnet et al., 2000).

Cadmium (Cd) is a widespread pollutant and one of the most toxic heavy metals in the environment due to its high mobility and toxicity at low concentration (Adriano, 1986; Farmer and Farmer 2000; Wagner, 1993). Cadmium contamination in soils has been reported to be the main constraint for food safety and agricultural land quality (Atafar et al.2010). Cadmium is an abiotic stress responsible proteins (Kamal et al. 2010).

Zinc (Zn) is an essential trace element for plants and animals, but is toxic when present at high levels. Cadmium and zinc are elements having similar geochemical and environmental properties; their chemical similarity can lead to interaction between Cd and Zn during plant uptake, transport from roots to the aerial parts, or accumulation in edible parts (Das et al. 1997). Antagonistic effects have been reported (McLaughlin and Singh 1999). It is generally accepted that Zn status in soils and plants plays an important role in Cd accumulation in crop plants (Grant and Bailey 1997; Oliver et al. 1997; Sarwar et al. 2010).

Interactions between Cd and Zn and their accumulation in plant parts in solution culture or in pot experiments have been reported (Coughtrey et al. 1979; Smilde et al. 1992; Mckenna et al. 1993; Moraghan 1993; Dudka et al. 1994; Long et al. 2003; Chizzola and Mitterenger 2005; Mohammad and Moheman 2009).

Increasing industrial production, utilization of fertilizers or natural sources may elevate content of heavy metals in the environment. This can be potentially dangerous for human health due to their bio-toxicity and high bioaccumulation throughout the food chain (Uraguchi et al. 2006). The objectives of this research were to examine the interactions of Cd and Zn and the effects on their respective

concentration in roots and shoots of pot *Solanum nigrum* L.

MATERIALS AND METHODS

Sampling site is situated at northern India at 25°57' N latitude and 81°50' E longitude on south-east facing slopes of comparable inclination at altitudes between 200 and 80m above sea level. A sandy clay loam, derived from Indo-Gangetic alluvial soils of SDI farm situated on the confluence of Ganga and Yamuna alluvial deposit, was sampled from Allahabad city, India. After a systematic survey, the experiment was laid out in factorial RBD design. The properties of the soil were: pH 7.8, EC 0.28 dSm^{-1}, organic matter ($K_2Cr_2O_7$ oxidation) 5.6 g kg^{-1}, total N 0.08%, total P 0.04%, CEC 19.8 Cmol (P^+) kg^{-1}, DTPA-Cd 0.38 mg kg^{-1} and DTPA-Zn 12.80 mgkg^{-1}. The texture was sand (>0.2 mm) 55.50%, silt (0.002-0.2 mm) 20.30% and clay (<0.002 mm) 24.20%. The detailed physicochemical properties of the investigated soil have been given in the Table 1. The soil was ground to pass through a 2 mm sieve. Plastic pots of a 5 litre capacity (each containing 5 kg of soil) were used. Fertilization added per kg soil was 0.8 g calcium ammonium nitrate, 0.5 g di-ammonium phosphate, 0.367 g potassium sulphate.

Zn was applied as $ZnSO_4.7H_2O$ to provide Zn at the rate of 0, 20, 40 and 60 mg kg^{-1}. Cd was applied as $CdCO_3$ at the rate of 0, 5, 10 and 15 mg kg^{-1} of soil with three replications of each treatment. Soil in each pot was mixed thoroughly to ensure intimate distribution of applied Cd and Zn. After 24 hrs of the treatments, seeds were sown. Soil moisture was maintained by irrigating the crops at intervals of 5-6 days. *Solanum nigrum* L. was grown a test crop.

Silt and clay were separated by Pipette method and fine sand by decantation. Di-ethyltriaminepenta acetic acid (DTPA) solution {1.97g (0.05M) DTPA powder, 13.3ml (0.1M) Tri-ethanol amine and 1.47g (0.01M) $CaCl_2$ were dissolved in distilled water made up to 1 litre after adjusting the pH to 7.3} was prepared (Lindsay and Norvell 1978) to extract the available heavy metals in soil samples. Five gram of soil was shaken with 20ml of the above reagent for 2

Table 1: Physicochemical properties of the Sheila Dhar Institute (SDI) Experimental Farm, Allahabad, India

Parameters	Values
Texture: Sandy Clay Loam (Sand, Silt and Clay %)	(55.50,20.30 and 24.20, respectively)
pH	7.8
EC(dSm^{-1}) at 25°C	0.28
Organic Carbon (%)	0.56
CEC [C mol (p^+) kg^{-1}]	19.8
Total Nitrogen (%)	0.08
Total Phosphate (%)	0.04
DTPA-extractable Cd (mgkg^{-1})	0.38
DTPA- extractable Zn(mg kg^{-1})	12.80

hr. The clean filtrate was used for the estimation of Cd and Zn by AAS at National Botanical Research Institute, Lucknow, India. Organic carbon was determined by chromic acid digestion method (Walkley and Black 1934) and CEC by using neutral 1N ammonium acetate solution. A known weight of soil (5g) is shaken with 25 ml of neutral ammonium acetate solution for 5 min and filtered through Whatman filter paper No. 42. For nitrogen a known weight of soil (1g) was taken in a 150 ml conical flask and treated with 10 ml of digestion mixture containing sulphuric acid and selenium dioxide. Salicylic acid was also added to include the nitrates and nitrites. Digestion was carried out till the soil colour changed to white. The N in the digest was estimated by steam distillation using micro-kjeldahl apparatus. For total P, the soil (2g) was taken with 4 ml $HClO_4$ (70%) in a 50 ml beaker covered with watch glass and put on a hot plate and digestion was carried till the soil colour changes to white.

Plants were harvested after 75 days. The green biomass was thoroughly sun dried and the dry matter yield was determined. Plant samples were digested in tri-acid mixture (750 ml conc. HNO_3, 150ml conc. H_2SO_4 and 300 ml $HClO_4$). Cd and Zn were determined by Atomic Absorption Spectrophotometer at National Botanical Research Institute, Lucknow, India. All data were statistically analyzed using Excel 2007 software.

Statistical analysis

Data were analyzed by factorial analysis of variation (ANOVA) using various treatments as independent factors with the help of the sum of square (SS) and degree of freedom (DF). The standard error (SE) is given by

$$SE = \sqrt{\frac{2V_e}{n}}$$

where, V_e is the variance due to the error, n is the number of replications, and the critical difference (CD) is given by $CD = SE_{diff} \times t_{5\%}$ ($t_{5\%}$= 2.042 at DF_{error} = 30 was observed) and standard deviation (SD) were determined in accordance with (Motulsky and Christopoulos, 2003).

RESULTS AND DISCUSSION

The data presented in table-2 (Fig -1) indicates almost highly significant effects of Cd, Zn and Cd ×Zn interaction on influencing the dry matter yield of *Solanum nigrum* L., which decreased as the doses of Cd increased up to 15 mg kg^{-1}. However, application on Zn up to 40 mg kg^{-1} either singly or in combination increased the dry matter content of all the pots, which resulted in 7.03 % extra dry matter yield over the control. But its higher level (beyond 60 mg kg^{-1}) antagonistically influenced the dry matter yield of the *Solanum nigrum* L. The Cd × Zn interaction was observed non-significant. The pronounced and diminutive effect on dry matter yield

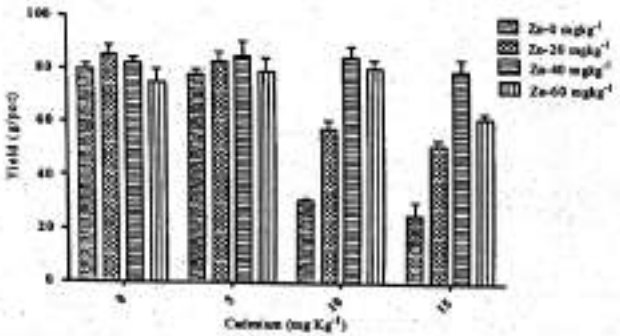

Fig.1: Effect of Cd × Zn interaction on dry biomass Yield of *Solanum*

was observed only in Cd (15 mg kg^{-1}) added pots which recorded 67.96% less over the control. Therefore, the response of Zn was observed ameliorative and encouraging in Cd contaminated pots (Gupta, et al. 1990). The adverse effect of Cd on the dry matter of *Solanum nigrum* L. was observed higher in magnitude than that of Zn. The decrease in yield may be due to reduced photosynthetic rate and internal water deficit in shoot system due to poor root development.

Clarkson and Lutge (1989) reported that Cd damages the biomembrane and cause enzymatic changes and possible interaction with macro and micro-elements leads to the phytotoxicity of this element. The decrease in yield with increasing rates of Cd application was also reported by Sarkunan et al. (1996) in rice; Georgieva et al. (1997) in radish, pea and peeper; Ozturk et al. (2003) in wheat.

The data presented in table-2 (fig 2 & 3) indicates that the effect of Cd, Zn and Cd × Zn interaction were observed almost highly significant. Accumulation of Cd in root and shoot of *Solanum nigrum* L. significantly increased as the doses of Cd increased up to 15 mg kg^{-1}. However, application of Zn antagonistically either singly or in combination reduced the Cd uptake in *Solanum nigrum* L. Application of Zn up to 60 mg kg^{-1} resulted in retarded or almost negligible accumulation of Cd in plants. Application of recommended doses of Zn in crops would be beneficial for combating Cd- toxification of plant (Gupta, et al. 1990). It appeared that application of Zn up to lower level (20 mg kg^{-1}) slightly increased the Cd uptake by shoots in some pots also. However, application of Zn antagonistically either singly or in combination reduced the Cd uptake in crop. Thus Cd and Zn interaction exhibited an antagonistic effect on

Table 2: Effect of Cd × Zn interaction on dry biomass yield and Cd concentration root and shoot of *Solanum nigrum* L.

Cd-rate (mg kg^{-1})	Zn-Source	Zn-rate (mg kg^{-1})	Yield (g/pot)	Cd-concentration (mg kg^{-1})	
				Root	Shoot
0	$ZnSO_4$	0	80.12	0.25	0.31
		20	85.81	0.53	0.65
		40	82.64	0.42	0.46
5	$ZnSO_4$	0	78.45	1.52	1.92
		20	83.78	1.13	1.42
		40	85.75	0.65	0.70
10	$ZnSO_4$	0	31.34	2.12	2.42
		20	58.69	1.72	2.08
		40	85.43	0.85	1.22
15	$ZnSO_4$	0	25.67	3.21	4.06
		20	52.35	1.75	2.10
		40	80.65	1.17	1.90
SE=			1.58	0.04	0.04
CD=			3.23	0.08	0.07

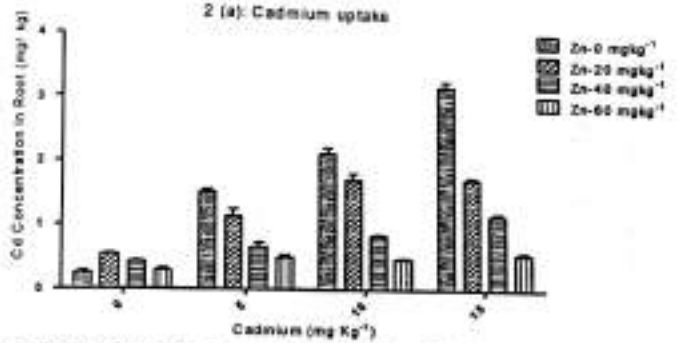

Fig.2: Effect of Cd × Zn interaction on uptake of Cd in root of *Solanum nigrum* L.

Cd concentration in roots of *Solanum nigrum* L. plants (Grejtovsky et al., Markusova et al. 2008).

This is to be expected, as Cd concentrations in plant tissue depend on both the genetic predisposition of the individual plant and the contamination levels of soil (Ma et al., 2001; Shah and Nongkynrih, 2007).

Cadmium and Zn might be considered chemically similar elements because they have similar ionic structure and electronegativities and may influence each other in plant uptake and accumulation, but they play quite different roles in the plants metabolism. Zinc is a micronutrient, whereas Cd is toxic and ordinarily is found at very low concentrations in the plant; usually, the Zn concentration is more than 100 times the Cd level (Chaney et al. 1999). However, they have different ionic radii (Zn^{2+} =0.074 nm, Cd^{2+} =0.097 nm);this difference may play a role in plant selectivity for Zn. In other words, the reduced uptake of Cd as a result of addition of Zn addition in our work might result from competitive transport and absorption interaction between these two ions. Zinc levels usually range between 20 to 100 mg kg^{-1} and maximum tolerable levels for Cd in agricultural soils proposed in various countries ranged from 150 to 300 mg Zn kg^{-1} (Kabata-Pendias and Pendias 1992).

Cd and Zn have almost similar ionic radii the simultaneous addition of both Cd and Zn reduced the adsorption of both ions. The interaction of Cd with Zn in plants is based on the substitution of Cd with Zn and decrease in Cd below its phytotoxic concentration in tissues (Purvis 1985, Kabatta-Pendias and Pendias 1989)

The interaction of Cd and Zn has been reported to be antagonistic by some researchers (Li et al. 1990; Long et al. 2003) but synergistic by others (Piotrowska et al. 1994; Salt et al. 1995; Nan et al. 2002). Perronnet et al. (2003) reported that Cd and Zn were distributed differently within the hyperaccumulating plant *Thlaspi caerulescens* and that the partitioning of these elements varied with plant age and organ. In wheat (*Triticum aestivum* L. and *T. turgidum* L. var. durum) at the level of the root cell membrane, Cd and Zn show a competitive interaction, indicating a common transport system (Hart et al. 2002). Various results have been reported concerning the interactions between the accumulation of Cd and Zn. Cadmium accumulation may or may not be influenced by increasing Zn supply. Great differences occur among species and even between different varieties of the same species (Grant and Bailey 1997). Some researchers found that Zn supply can inhibit Cd adsorption and thereby cause a low Cd concentration in plants (Adriano 1986; Nan et al. 2002). Results from the present work showed that Cd concentration in the studied plant tissues of *Solanum nigrum* L. were largely dependent on the Zn level. Cadmium concentration in roots and shoots

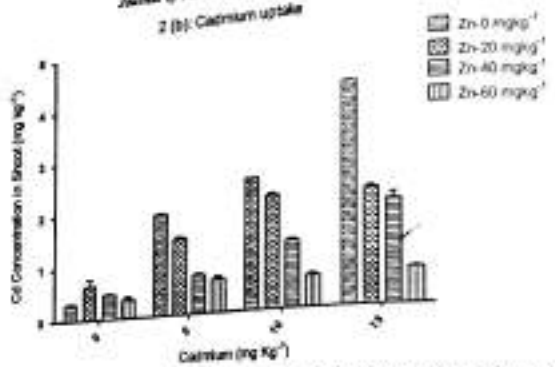

Fig.3. Effect of Cd × Zn interaction on the uptake of Cd by shoot of *Solanum nigrum L.*

decreased significantly with increasing Zn application to the soil (Fig. 2&3).

CONCLUSION

The dry biomass yield of *Solanum nigrum* L. was decreased (67.96%) with the single application (Cd 15mgkg^{-1}) and increased (7.03 %) with the combinatorial application (Cd 5 + Zn 40 mgkg^{-1}) compared to control.

The results indicate that the single application of Cd 15mg kg^{-1} soil enriched the content of Cd up to 1184% (3.21mgkg^{-1}) in roots and up to 1209.68% (4.06mgkg^{-1}) in shoots of *Solanum nigrum* L. compared to control. When used combinatorial application (Cd 15 + Zn 60 mg kg^{-1}) soil decreased the content of cadmium up to 124% (0.56mgkg^{-1}) in root and up to 106% (0.64mgkg^{-1}) in shoots of *Solanum nigrum* L.

The reduced uptake of Cd was observed in zinc treated plots. An ameliorative effect of zinc was observed in Cd-contaminated soil. The results of presented study showed that Zinc can effectively immobilize Cd in the soil. Zinc has potential to reduce Cd accumulation in both root and shoot of the *Solanum nigrum* L.

The application of Zn to the soil possibly reduces Cd in the edible parts of the plants and helps to reduce the risk to the health of people living in metal contaminated areas. A more detailed study is required to grow *Solanum nigrum* L. or other vegetable crops in metals- contaminated areas and evaluate their growth and distribution of heavy metals in different edible parts of plants.

In view of the uncertainties that remain about the behavior and effects of Cd in the food chain, it is desirable to minimize its concentration in crops that are grown on sewage- irrigated soils.

As the uptake of Cd is reduced in presence of Zn, a clear antagonism takes place. The addition of Zn is bound to decrease the uptake of Cd by the *Solanum nigrum* L. Where there is an access of industrial effluent rich in Cd, such amendments can be of practical value.

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REFERENCES

Adriano DC., (1986). Trace elements in the terrestrial environment. Springer- Verlin.

An, Y, J., (2004). Soil ecotoxicity assessment using cadmium sensitive plants. Environmental Pollution 127, 112-119

Ataie, Z.; Mesdaghinia, A.; Nouri, J.; Homaee, M.; Yunesian, M.; Ahmadimoghaddam, M. and Mahvi H, (2010). Effect of fertilizer application on soil heavy metal concentration. Environ Monit Assess 160:83-89.

Chaney, RL.; Ryan, JA.; Li YM and Brown S.L. (1999). Soil cadmium as a threat to human health. In; McLaughlin MJ and Singh B R (eds) Cadmium in soils and plants, pp 219-246. Kluwer Academic Publishers: Dordrecht, the Netherlands.

Clarkson, D.T.; Lutge U, (1989) III. Mineral nutrition: divalent cations, transport and compartmentation. Progress in Botany 51: 93-112.

Chizzola, R. and Mitterenger, U. (2005). Cadmium and zinc interactions in trace element accumulation in chamomile. J. Plant Nutr 28: 1383-1396.

Coughtry, P. J.; Jones, C.H.; Martin, M.H. and Shales, S.W. (1979). Litter accumulation in woodlands contaminated by Pb, Zn, Cd, and Cu Oecologia 39:51-60.

Das, P.; Samantaray, S. and Rout, G.R., (1997). Studies on cadmium toxicity in plants: a review. Environ Pollut 98:29-36.

Dudka, S.; Piotrowska, M. and Chlopecka, A. (1994). Effect of elevated concentration of Cd and Zn) In soil on spring wheat yield and the metal contents of the plants. Water Air Soil Poll.76:333-341.

Farmer, A.A. and Farmer, A.M. (2000). Concentration of cadmium lead and zinc in livestock feed and organs around a metal production center in eastern Kazakhstan Sci. Total Environ 257:53-60.

Georgieva, V.; Tasev, C. and Senalevitch, G. (1997). Growth, yield, lead, Zn and Cd cotent of radish, pea and pepper plants as influenced by level of single and multiple contamination of soil. III. Cd. Bulg. J. Plant Physiol. 23: 12-23.

Ghosh, M. and Singh, S. P. (2005). A comparative study of cadmium phytoremediation by accumulator and weed species. Environmental Pollution 33, 365-371.

Grant, C.A. and Bailey, L.D. (1997). Effects of phosphorous and zinc fertilizer management on cadmium accumulation in flaxseed. J. Sci. Food and Agric 73:307-314.

Grejtovsky, K.; Markusova and Novakova, L. L. (2008). Plant Soil Environ, 52, (2), 47-54.

Gupta, V.K. and Potalia, B.S.,(1990). *J. Indian Soc. Soil Sci.*, 38, 452-457.

Hart, J.J.; Welch, R.M.; Norvell, W.A. and Kochian, L.V. (2002). Transport interaction between cadmium and zinc in roots of bread and durum wheat seedlings. Physiologia Plantarum 116: 73-78.

Kabatta, Pendias, A. and Pendias, H. (1989). Trace elements in soils plants. Mir. Moscow., 152-186.

Kabata, Pendias A. and Pendias, H. (1992). Trace elements in soils and plants, 2nd edition, Levis Publ. Inc. 365pp.

Kamal, A.H.M.; Kim, K.H.; Shin, K.W.; Choi, J.S.; Baik, B.K.; Tsujimoto, H.; Heo, H.Y.; Park, C.S. and Woo, S.H. (2010). Abiotic stress responsive proteins of wheat grain determined using proteomics technique. Aust. J. Crop Sci. 4:196-208.

Li, S.L.; Wang, H.X. and Wu, Y.S. (1990). Antagonistic effect of zinc on cadmium in water hyacinth. Acta Scientiae Circumstantiae 10: 244-249.

Lindsay, W. L. and Norvell, W.A. (1978). Soil Sci. Soc. Am J., 42, 421.

Long, X.X.; Yang, X.E.; Ni, W.Z.; Ye, Z.; He, Z.L.; Calvert, D.V. and Stoffels, J.P. (2003). Assessing zinc thresholds for phytotoxic and potential dietary toxicity in selected vegetable crops. Commun Soil Sci. Plant Anal 34:1421-1434.

Ma, L.Q.; Komar, K.M.; Tu, C.; Zhang, W.; Cai, Y. and Kennelley, E.D. (2001). A fern that hyperaccumulates arsenic, Nature 409, 579.

Mckenna IM, Chaney RL, Williams FM, 1993. The

effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. Environ Pollut 79:113-120.

Mclaughlin MJ, Singh BR, 1999. Cadmium in soils and plants Kluwer Academic Publishers: Dordrecht, the Netherlands, pp. 257-267.

Mohammad A, Moheman A, 2009. The effects of cadmium and zinc interaction on the accumulation and tissue distribution of cadmium and zinc in tomato (*Lycopersicon esculentum* mill.). Arch of Agron Soil Science 56(5): 551-561.

Monaghan JT, 1993. Accumulation of cadmium and selected elements in flax seed grown on a calcareous soil. Plant Soil 150:61-68.

Motulsky H. J. and Christopoulos A., 2003. GraphPad Software Inc, San Diego CA.

Nan Z, Li J, Zhang J, Cheng G, 2002. Cadmium and zinc interactions and their transfer in soil crop system under actual field conditions. Sci. Total Environ 285: 187-195.

Oliver DP, Wilhem NS, McFarlan JD, Tiller KG, Cozens GD, 1997. Effect of soil and foliar applications of zinc on Cd concentration in wheat grain. Aust. J. Exp. Agric. 37:103-107.

Ozturk L, Eker S and Ozkutlu F, 2003. Effect of Cd on growth and concentration of Cd, Ascorbic acid and sulphydryl groups in durum wheat cultivars. Turk J Agric For 27: 161-168.

Perronnet, K., Schwartz, C., Gerard, E., Morel, J.L., 2000. Availability of cadmium and zinc accumulated in the leaves of *Thlaspi caerulescens* incorporated into soil. Plant Soil 227, 257-263.

Perronnet K., Schwartz C., Morel JL, 2003. Distribution of cadmium and zinc in the hyperaccumulator *Thlaspi caerulescens* grown on multicontaminated soil. Plant Soil 249: 19-25.

Piotrowska M, Dudka S, Chilopecka A, 1994. Effect of elevated concentrations of Cd and Zn in soil on spring wheat yield and metal contents of plants. Water Air Soil Pollut 76: 333-341.

Purvis D., 1985. Trace element contamination of the environment. Elsevier, Amsterdam-oxford-New York-Tokya, 8-147.

Salt DE, Blaylock M, Kumar NBP, 1995. Phytoremediation: a novel strategy for the removal of toxic elements from the environment using plant. Biotechnology 13: 468-474.

Sarkunan V, Mishra A K and Mohapatra A R 1996. Effect of Cd and Zn on yield and Cd and Zn content in rice. J Ind Soc Soil Sci 44: 346-48.

Sarwar N, Saifullah, Sukhdev SM, Munir HZ, Asif N, Sadia B, Ghulam F, 2010.Role of mineral nutrition in minimizing cadmium accumulation by plants (Review). J. Sci. Food Agric 90:925-937.

Shah,K., Nongkynrih, J.M., 2007.Metal hyperaccumulation and bioremediation. Biologia Plantarum 51(4), 618-634.

Shenker M, Fan T W M and Crowley D. E., 2001. Phytosiderophores influence on Cd mobilisation and uptake by wheat and barley plants. J. Environ. Qual. 30:2019-98.

Smilde K W, van Luit B, van Driel W, 1992. The extraction by soil and absorption by plants of applied zinc and cadmium, Plant and Soil 143:233-238.

Uraguchi S., Watanabe I., Yoshitomi A., Kiyono M., and Kuno K., 2006. *J. Exp. Bot.*, 57, 2955-2965.

Wagner G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv Agron 51:173-213.

Walkley A. and Black I.A. 1934. Organic carbon was determined by chromic acid digestion method. Soil Science 37, 29-38.

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EFFECT OF ZINC SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY OF LEAD FED GROWING KIDS

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ABSTRACT

A study was conducted to find out the effect of zinc supplementation on nineteen crossbred goat kids (Alpine x Beetle) exposed to 50 ppm lead. The animals divided into three groups (Control, T_1 and T_2) were fed concentrate mixture and green Lucerne (NRC, 1981). In T_1 group, kids were given 50 ppm of lead and T_2 animals were supplemented with 50 ppm lead+50 ppm of zinc daily for 90 days. The DMI/ 100 kg body weight were 2.20, 2.43 and 2.39 in control, T_1 and T_2 groups respectively (Pe"0.05). The dietary treatments did not have any adverse effect (Pe"0.05) on the body weight gain and the digestibility of OM, CP, EE, NDF and ADF. The DM digestibility in control, T_1 and T_2 groups was 49.51±1.57, 45.15±0.81 and 47.95±1.01 per cent respectively showing a significant effect of dietary treatments (P<0.05). Results indicated that Zn can be helpful in mitigating the adverse effect of lead

Keywords: Lead, zinc supplementation, digestibility, kids

Livestock is exposed to a wide range of toxicants from various sources (Radostits et al., 2005). Among the toxicants heavy metal like lead, arsenic, cadmium, mercury etc. are widespread and detrimental to animal health. In India lead toxicosis of livestock has been reported from five states viz., Punjab, Delhi, Rajasthan, Andhra Pradesh, and Maharashtra (Dogra et al, 1996).Lead either alters the liver functions directly by binding with thiol group of liver enzyme and some carrier proteins (Jones, 1954) or metabolized to more toxic product and free radicals, which alters the mitochondrial activity and genetic information. Zinc has a protective effect in lead exposed animals as it interferes with the absorption of lead as zinc and lead compete for similar binding sites on the metallothionein like transport protein in the gastrointestinal tract, thus reducing lead toxicity. The information regarding the supplementation of zinc to lead exposed animals to counteract the adverse effect is scanty. Thus, the present study was carried out to observe the protective effect of zinc on the adverse effect of lead by studying the dry matter intake (DMI), nutrient utilization parameters, and growth rate and blood antioxidant enzymatic profile in growing crossbred kids.

MATERIALS AND METHODS

Nineteen Alpine Beetle crossbred male goat kids were selected from the institute herd. The animals were randomly divided into three groups (control-C, T_1 with 50 ppm lead as lead acetate supplementation and T_2 with 50 ppm zinc in addition to 50 ppm lead) with initial body weight (kg) of 8.92, 9.14 and 8.93 kg. The nutrient requirement of the animals was met by feeding concentrate mixture (CP 20% and TDN 70%) and lucerne as per NRC (1981). The concentrate mixture comprised of GNC 21 parts, Maize 33 parts, wheat bran 20 parts, rice bran 11 parts, de-oiled mustard cake 12 parts, mineral mixture 2 parts and common salt 1 part. Calculated amount of lead and zinc were weighed and put into a gelatin

capsule. This capsule was given to the animal orally to ensure that the animal has consumed the required quantity. The ration schedule was changed weekly after recording the body weight of the animal to meet the nutrient requirement. The clean drinking water was offered three times a day i.e. at 8:00 am, 12:00 noon and 6:00 pm after three months of feeding, a digestibility trial of 5 days collection was conducted to determine the digestibility of different nutrients. The animals were weighed before and after conducting digestion trial. During digestion trial, weighed amount of concentrate mixture and luceme were offered daily to animal and the weight of the residue was taken everyday to record the intake. The sample of feeds i.e. concentrates mixture and luceme offered and residue left were taken for chemical analysis. The animals were offered water twice in a day i.e. morning and evening. The quantity of feaces excreted by animals during 24 hr period was recorded for 5 days. The feaces was collected in a bucket of respective animal, was thoroughly mixed and representative sample of feaces was taken to laboratory for chemical analysis.To determine the DM and proximate principles of dung, an aliquot of 1/100 of total faeces voided daily was taken individually for each animal. The 5 days feaces samples were pooled from respective animal in separate polythene bags. However, for nitrogen estimation in faeces, an aliquot of 1/500 of fresh feaces sample was taken separately in a plastic container. To preserve the faecal sample, 10 ml of 25% H_2SO_4 was added to each plastic container. The containers in which the aliquots of dung for nitrogen estimation had been kept were weighed before and after the trial for accurate quantification of nitrogen voided through dung. At the end of 5 days collection period, samples were mixed thoroughly and 5 g sample were taken for nitrogen estimation. The samples of feed offered to animals i.e. concentrate mixture, Lucerne were analyzed for proximate principles viz. dry matter (DM), crude protein (CP), ether extract (EE) and total ash as per standard procedure of Association of Analytical Chemists. Cell wall components (NDF), lingo-cellulose component (ADF), and Acid detergent lignin were estimated by the method of Goering and Von Soest (1970). All the data was subjected to the statistical analysis as per the analysis of variance (ANOVA) technique (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

A digestibility trial of 5 days duration was conducted after 14 weeks of feeding the dietary treatments. During this period, the DM intake (g/d) of the kids was 406.24±56.07, 390.90±34.70 and 393.42±42.57 in control, T_1 and T_2 groups

Table 1: Effect of zinc supplementation on nutrient digestibility in lead fed goats

Particulars	Group			Significance
	Control	T_1	T_2	
Digestibility Coefficients				
DM	$49.51^{a} \pm 1.57$	$45.15^{b} \pm 0.81$	$47.95^{ab} \pm 1.01$	S
OM	54.33 ± 1.62	52.03 ± 1.23	52.86 ± 1.65	NS
CP	52.79 ± 0.10	50.09 ± 0.76	50.91 ± 0.34	NS
EE	65.60 ± 0.99	63.93 ± 0.91	64.21 ± 1.23	NS
NDF	46.97 ± 1.14	43.99 ± 1.06	45.83 ± 0.96	NS
ADF	36.81 ± 1.43	34.58 ± 1.28	35.15 ± 1.11	NS

respectively. The DM intake through concentrate was 209.24±24.91, 199.50±62.0 and 201.64±22.49 g/d whereas through green Lucerne it was 197.33±46.40, 190.81±11.75 and 192.01±18.20 g/d in three groups respectively. The digestibility coefficients of various nutrients are presented in Table 4. 7 The DM digestibility in control, T_1 and T_2 groups was 49.51±1.57, 45.15±0.81 and 47.95±1.01 per cent respectively showing a significant effect of dietary treatments (P<0.05). The digestibility of OM, CP, EE, NDF and ADF were not influenced significantly (Pe" 0.05) by lead or lead +zinc supplementation in goat kids. Value bearing different superscripts in a row (a, b) differ significantly (P<0.05).

The nutrient digestibilities of all the proximate principles and cell wall constituents in T_1 group (lead supplemented group), were lower as compared to control group. It indicated that dietary lead supplementation had an adverse effect on nutrient digestibility. Lead has a toxic effect on rumen fermentation as explained earlier under *in vitro* experiments in the present study. The results obtained in the present studies are in close agreement to those reported by Dinius *et al.* (1973) who did not find any significant effect on digestibility of DM, protein and energy after lead supplementation. However, in their studies ADF digestibility decreased significantly with increased dietary lead levels. In the present studies, though, there was decrease in nutrient digestibilities of all the parameters in lead exposed kids (T_1), but the result were insignificant statistically (Pe" 0.05). Arvind (2003) and Fick *et al.* (1976) also did not find any effect of lead exposure on nutrient digestibility of DM, OM and ADF in calves and sheep exposed to dietary lead levels of 50 ,100 ppm and 1000 ppm in diets. However, Arvind (2003) obtained an adverse effect of lead exposure on EE digestibility. But in our studies no such effect was observed.

CONCLUSION

The DM digestibility in control, T_1 and T_2 groups was 49.51±1.57, 45.15±0.81 and 47.95±1.01 per cent respectively showing a significant effect of dietary treatments (P<0.05). The digestibility of OM, CP, EE, NDF and ADF were not influenced significantly by lead or lead +zinc supplementation in goat kids (Pe"0.05).

REFERENCES

Arvind Kumar, (2003). Dietry lead levels influencing growth, milk yield, blood serum profile, and lead excretion pattern in ruminants. PhD. Thesis submitted to NDRI, Karnal (Reg. No. 1089902).

Dinius, D.A.; Brinsfield, T.H. and William, E.E. (1973). Effect of subclinical lead intake on calves. *J. Anim. Sci.*, 37:169-173.

Dogra, R.K.S.; Murthy, R.C.; Srivastava, A.K.; Gaur, J.S.; Shukla, L.J. and Varmani, B.M.L. (1996). Cattle mortality in the Thane district, India A study of cause/effect relationship. *Archiv. Environ. Contam. Toxicol.* 30:292-297.

Fick, K.R.; Ammerman, C.B.; Miller, S.M.; Simpson, C.F. and Loggins, P.E. (1976). Effect of dietary lead on performance, tissue mineral composition and lead absorption in sheep. *J. Anim. Sci.*, 42:515-523.

Goering, H.K. and Vansoest, P.J. (1970). Forage fibre analysis (Apparatus, reagents, procedures and same applications) ARS US Deptt. Handbook No. 379, Washington, DC.

Jones, L.M. (1954). In: Veterinary Pharmacology and Therapeutics. Ames: Iowa State College Press

NRC, (1981). Nutrient requirements of goats in temperate and tropical countries. *Nat. Acad Sci., Washington D.C.*

Radostits, O.M.; Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2005). Veterinary Medicine, 9th Edition, Bailliere Tindal, London, pp. 1575

Snedecor, C.W. and Cochran, W.G. (1994). Statistical method. Iowa State University press, *Ames, Iowa.*

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UTILIZATION OF SWEET POTATOES IN THE PREPARATION OF SWEET PRODUCTS

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ABSTRACT

Sweet potato was incorporated in khoa based sweet products at three different level 25 per cent (T_1), 50 per cent (T_2) and 75 per cent (T_3). The products were subjected to organoleptic analysis by the panel of five judges. The products were scored on the basis of Nine-point Hedonic scale. Based on organoleptic attributes product *burfi* was highly accepted in T_3 (75 percent sweet potato and 25 percent khoa) and in gujia T_2 (50 percent sweet potato and 50 percent khoa) was highly acceptable and gulabjamun T_1 (25 percent sweet potato and 75 percent khoa) also acceptable.

***Key words:** Khoa, sweet potato, refined flour*

The sweet potato or "shakarkand" (*Ipomoes Batatas*) are rich in starch, which are small in size. The important constituents of fresh tubers are carbohydrate, fat, sugar and appreciable amount of human diet. They are plant or parts of plant that are used as a food. Vegetables are important in improving the acceptability of the meal, because of innumerable shades of color, flavor and texture they contribute. The nutritive value of sweet potato (amount of nutrient in 100g of sweet potato) is: Protein-1.2g, Fat- 0.3g, Minerals-1.0g, Carbohydrate- 8.2g, Energy- 120g, Manganese- 0.22g, Calcium- 46mg, Phosphorus-50mg, Iron- 0.21g, Carotene- 6µg, Thiamin- 0.08mg, Riboflavin- 0.10mg, Niacin- 0.3mg, Zinc- 0.11mg, vitamin C- 24mg, Sodium- 9.0mg, Potassium-393mg, Copper-0.02mg, Chromium- 0.006mg. Approximate total nitrogen content in gram per 100 gram sweet potato is 0.19g. Essential amino acid content of sweet potato in mg per 100 gram of nitrogen in sweet potato is: Ariginine-280, Histidine- 90, Lysine-260, Tryptophan- 110, Phenyl alanine- 270, Tryosine-150, Methionine- 100, Cystein- 30, Threonins- 280, Leucine- 360, Isoleucine- 290 and Valine- 380 (Gopalan et al. 2002).

Sweet potato deteriorates rapidly and deteriorations start within 6 weeks of harvesting. Converting fresh sweet potato into value added products like sweet potato and products made by them would provide farmers, better way to increase profit, provide nutrition and also to make availability in off season. Despite its name, the sweet potato is not related to the potatoes. Potatoes are member of the solanaceae family while sweet potatoes belong to the morning-glory family (convolvulaceae). It requires warm temperature, plenty of sunshine and moderate rainfall for growth and can be harvested in three to six months.(Chattopadhyay et. al., 2005)

According to CIP (International Potato Centre) sources yellow, orange fleshed varieties of sweet potatoes are now being used in Africa to combat a widespread vitamin A deficiency that result in blindness and even death for about five million African children a year. The CIP believes there is a good potential for improving the diet and thereby, a child's nutritional status by incorporating sweet potato into the diet.

MATERIALS AND METHODS

The present study was carried out in the Nutrition research Laboratory of the Foods and Nutrition, Ethlind School of Home Science, Sam Higginbottom

Institute of Agriculture Technology & Sciences (Deemed to be University) Allahabad during 2010-2011.

1. **Procurement of raw materials:** Sweet potatoe and other products (Flour, refined oil, semolina, sugar, cardom, baking powder and khoa) were collected from the the local market of Allahabad in the month of January 2010.

2. **Development of foods products:** Three food products namely *Burfi, Gujia* and *Gulabjaman* were developed by incorporating fresh sweet potato at different three levels.

3. **Treatments and replications of products:** The basic standard recipes were served as control (T_0). Three treatments, i.e., incorporation of sweet potato at three different levels were referred to as T_1, T_2 and T_3 respectively for each of the three products made.

3.1 **Details of treatments:**

a) **T_0 (control):** In this, the products were prepared with only the standard recipes (khoa based) without any incorporation of sweet potato.

b) **T_1 (25%, 75%):** In this treatments, 25 percent sweet potato was incorporated in 75 percent khoa for making *burfi, gulabjaman* and *gujia*.

c) **T_2 (50%, 50%):** 50 percent sweet potato and 50 percent khoa were used for making *burfi, gulabjamun* and *gujia*.

d) **T_3 (75%, 25%):** 75 percent sweet potato was substituted and used in 25 percent khoa for making *burfi, gulabjamun* and *gujia*.

4. **Organoleptic analysis of the cooked products:** The organoleptic characteristics of the developed products were evaluated using 9 point hedonic scale, by five panel members randomly selected from the Ethlind School of Home Science.(Bedi et. al., 2006).

5. **Statistical method:** Data is as curtained from the experiment was statistically analysed using analysis of variance technique and critical difference test.

RESULTS AND DISCUSSION

Data revealed that the sweet potato can be suitable incorporated in khoa based sweet products (*burfi, gujia and gulabjamun*). Sensory scores of *burfi* revealed that T_3 (75percent) was liked very much while T_0 (control), T_1 (25percent), T_2 (50 percent) were liked moderately by the panel of judes. Sweet potato incorporate *gujia* at T_2 (50percent).

Table 1 : Treatments and replications of products

Treatments	Products and incorporation levels of sweet potato		
	Burfi	Gujia	Gulabjamun
T_0(control)	-	-	-
T_1	25%	25%	25%
T_2	50%	50%	50%
T_3	75%	75%	75%

Table 1: Effect of incorporation of sweet potato on different parameters of *Burfi*. Average sensory scores of different parameters in control and treated sample of *Burfi*.

Treatments	Color	Texture	Flavour	Taste	Over all acceptability
T0	7.6	7.52	7.72	7.76	7.65
T1	8.08	7.64	7.80	8.00	7.88
T2	7.56	7.64	7.72	7.84	7.69
T3	8.56	8.44	8.60	8.52	8.54
F-test	15.7	14	15.16	5.52	17
CD	0.34	0.34	0.32	0.43	0.30
S/Ns	S	S	S	S	S

Table 2: Effect of incorporation of sweet potato on different parameters of *Gujia*. Average sensory scores of different parameters in control and treated sample of *Gujia*.

Treatments	Color	Texture	Flavour	Taste	Over all acceptability
T0	7.72	7.76	7.56	7.64	7.67
T1	8.60	8.52	8.36	8.52	8.50
T2	8.80	8.64	8.68	8.84	8.74
T3	8.52	8.44	8.28	8.24	8.37
F-test	4	2.22	5.6	4.60	4.56
CD	0.71	0.80	0.61	0.71	0.65
S/Ns	S	Ns	S	S	S

Table3: Effect of incorporation of sweet potato on different parameters of *Gulabjaman*. Average sensory scores of different parameters in control and treated sample of *Gulabjamun*.

Treatments	Color	Texture	Flavour	Taste	Over all acceptability
T0	7.98	7.36	7.48	7.50	7.38
T1	8.40	8.64	8.80	8.76	8.65
T2	7.76	7.88	7.88	7.88	7.85
T3	7.48	7.24	7.20	7.24	7.29
F-test	4.35	7.25	12.7	11	11.35
CD	0.76	0.71	0.58	0.61	0.56
S/Ns	S	S	S	S	S

Sensory scores of gulabjamun T_1 (25 percent) score maximum followed by T_0 (control), T_2 (50 percent) and T_3 (75 percent) respectively.

CONCLUSION

Incorporate products of burfi, gijia and gulabjaman showed a consumer acceptability and a reduced cost of production, therefore a incorporation of sweet potato in recipes of daily diet can be recommended to the poorer section of the community in order to improve their nutrients intake, particularly carbohydrate, vitamin A and vitamin C as well economize on the cost.

REFERENCES

Bedi, R.; Sadnan B. and Verma S. (2006) "Effect of cooking methods on the mineral availability for commonly consumed vegetables." *Journal of Food Science and Technology*, 43(5): 512-514.

Chattopadhyay, A.; Chakraborty, I.; Kumar, P.R.; Nanda, M.K. and Sen, H. (2005) "Uncontrolled storage of sweet." *Journal of Food Science and Technology*, 43(1): 41-44.

Gopalan, C.; Ramasastri, B.V. and Balasubramanian, S.C. (2002) "Nutritive value of Indian Foods." NIN ICMR, Hydrabad 50-61.

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EFFECT OF DIFFERENT LEVELS OF MANGANESE ON PERFORMANCE OF BROILERS

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ABSTRACT

Data indicated that a significant effect of dietary inclusion of manganese on the Body Weight and Gain in Weight and Feed Intake of the caged broilers. However the FCR of caged broilers was not significantly influeaced by dietary inclusion of manganese in the ration of broiler chicken.

Key words: Broilers, levels, manganese.

People in our country suffer mostly from protein inadequacy because of insufficient availability of milk, meat, egg and fish which are the best source of quality protein for human being. According to modern concept the daily protein requirement of an adult being are adequately met if the feed provides about 1 gm of protein per Kg of body weight. Minimum protein of healthy Indian has been fixed at 55-60 gm per day by the nutrition expert of (ICMR) of which about 30% (i.e.15gm) should be from animal protein Gopalan et al.,(1981) where as availability is claimed to be g per day. Broiler production plays a major role in food security for rapidly increasing human population. Their short production cycle, high feed efficiency and high biomass perunit of agricultural land are particularly attractive for the world production system.However, compared to other domestic animals, broiler chickens are more susceptible tochanging environmental conditions Nolan et al.(1999).

A deficiency of manganese in the diet of young growing chickens is one of the causes of perosis and of thin-shelled eggs. Most poultry feedstuffs are poor sources of manganese. Perosis caused by manganese deficiency is exacerbated by excess calcium and phosphorus in the diet. Birds reared on wire or slatted floors are more susceptible to perosis than those reared on litter. All commercial poultry diets are now supplemented with a source of available manganese(manganese sulfate). Because manganese deficiency is now rare, other possible causes should be considered when perosis isencountered hatchability 0f broiler chicks are more susceptible to changing environmental conditions (Nolan et al.1999).

MATERIALS AND METHODS

The present study was carried out at Sundaresan School of Animal Husbandry and Dairying, SHIATS, Allahabad on 15 day-old broiler chicks (DOC) which were randomly divided into five groups. Day old 15 broiler chicks of same hatch were procured and housed in battery type cages consisting of three chicks in each to provide recommended floor space of 0.75ft² in cage per broiler in small animal laboratory of the Sundaresan School of animal husbandry and dairying, SHIATS. Chicks were provided with self-prepared ration as per following treatments combinations:

T0= control
T1= T0+ 30mg/Kg manganese.
T2= T0+ 60mg/Kg manganese.
T3= T0+ 90mg/Kg manganese.
T4= T0+ 120mg/Kg manganese.

The standard broiler starter ration containing CP: 22 and, ME:2900 and broiler finisher ration containing

CP: 19 and ME:3000 were fed *ad lib* to the birds as per BIS (1992).

STATISTICAL ANALYSIS:

Data on various parameters (weekly feed intake & FCR) were collected, tabulated and analyzed statistically using analysis of variance techniques as per Snedecar and cochran(1994).

RESULTS AND DISCUSSION

Average body weight of broiler:

In general the body weight of day old broiler chicks ranged from 42 to 42.6g.The mean bodyweight of DOC in different treatment viz. T0 to T4 was 42.6,42,42.6, 42, and 42g, respectively. The differences in mean body weight of the chicks were not significant. The results pertaining to the body weight of DOC contained in Table 2 and Fig 1 indicated that broiler in T0, T2 had the highest body weight 42.6(g) but it did not significantly differ from other treatments. AndIrrespective of treatments in general the body weight of the broilers at fifth weeks of age ranged from 925 g to 1141g. The mean body weight at fifth weeks of age in different treatments viz.T0 to T4 were 953.3, 1046.6,1082.3,1139,1141.3, respectively. The differences in mean body weight of the broilers of fifth weeks of age between treatments were significant Table1.Highest mean body weight of broilers at fifth weeks of age was recorded in T4 (1141.3g) followed by T4. And lowest mean was observed in T0 (953.3g). The differences in these values of body weights were found significant indicated there by significant effect of treatments on body weight of broilers. The body weight of broilers at fifth weeks of age in T1, T2, T3 and T4 were found non-significant being at bar. The control (T0) is the lowest treatments in body weight of broilers at fifth weeks of age registered significantly compared to all treatments.. The results tally with the findings of Ozkan et al. (2003) and Balog et al., (2003).

Table 1: Ingredients and nutrients composition of experimental diet:

Ingredients (%)	Broiler starter(0 – 21 days)	Broiler finisher(0– 21 days)
Maize	60.00	63.00
Ground nut cake	23.35	18.00
Fish meal	13.00	15.00
Mineral mixture	3.00	3.00
Common salt	0.05	0.38
Vitamin premix (vit A , B2,D3)	0.05	0.02
Nutrient composition		
Moisture (%)	6.29	6.22
Crude fibers (%)	5.50	6.00
Total ash (%)	8.02	9.34
Crude protein (%)	22	19
ME(Kcal\kg)	2900	3000

Average gain in weight (g) of broiler:

In general average gain in weight of the broilers at 5 weeks of age ranged from 910.4 g to 1099 (g).The mean gain in weight at 5 weeks in different treatments viz. T0 to T4 was 910.7,,1004, 1039,1097 and 1099 g respectively. The differences in mean gain in weight of the broilers of 5 weeks of age between treatments were significant Table2& Fig1. The highest mean gain in weight of broilers at weeks of was recorded in T4 (1099g). And the lowest T0 (910.7g). However differences in these values of gain in weight were found significant indicated there by significant effect of treatments on gain in weight of broilers. Gain in weight of broilers at 5weeks of age in T1, T2, T3 and T4 were not significant being at bar. The control T0 registered significantly the lowest gain in weight compared to all the treatments. The results tally with the finding of Sobayo (2005) and Beck, (1991) and Smith et al,(1995).

Average feed intake (g) of broilers

In general average feed intake of the broilers at 5weeks of age ranged from 1750 g to 2254.5 g.The mean feed intake at 5 weeks of age in different treatments viz. T0 toT4 was 1750, 2039, 2060.8, 2254.5 and2208.3g, respectively. The differences in mean feed intake of the broilers of 5weeks of age between the treatments were significant Table2& Fig1 indicating thereby a significant effect of treatments on feed intake of broilers. The highest mean feed intake of broilers at 5 weeks of age was recorded in T3 (2254.5g) and the lowest in T0 (1750g).However Feed intake of broilers at 5weeks of age in T1, T2, T3, and T4 were not significantly different 5% level being at par. The broilers in control (T0) registered significantly lowest feed intake compared to all treatments. The results tally with the finding of Beck (1991) and Takahashi et al., (1991).

Average feed Conversion Ratio (Kg) of broilers:

In general the FCR of the broilers ranged from 1.92 to 2.0 .The mean FCR (feed required for per kg gain in weight) in different treatments viz.T0 toT4was1.92, 2.0, 1.98, 2.0and 2.0, respectively. The differences in mean FCR of the broilers were not significant indicating thereby a non-significant effect of dietary inclusion of manganese in the ration of broiler chicken. The results pertaining to the FCR contained in Table 2& Fig1 indicated that broiler in T0 registerod the best FCR compared to all other treatments; however it was not significantly different from other treatments being at par. The results tally with the findings of Smith et al, (1995).andTakahashi et al., (1991)

Table 2 : Mean values different parameters:

Treatments	Parameters				
	body weight of DOC age(g)	body weight at 5th week of age(g)	Gain in weight in 5 weeks (g)	Feed Intake in 5 weeks(g)	FCR in 5 weeks
T0	42.6	953.30	910.7	1750	1.92
T1	42	1046.60	1004.6	2039	2.0
T2	42.6	1082.30	1039.7	2060	1.98
T3	42	1139.00	1097	2254.5	2.0
T4	42	1141.30	1099.3	2208.3	2.0
Results	**NS	*S	S	S	NS

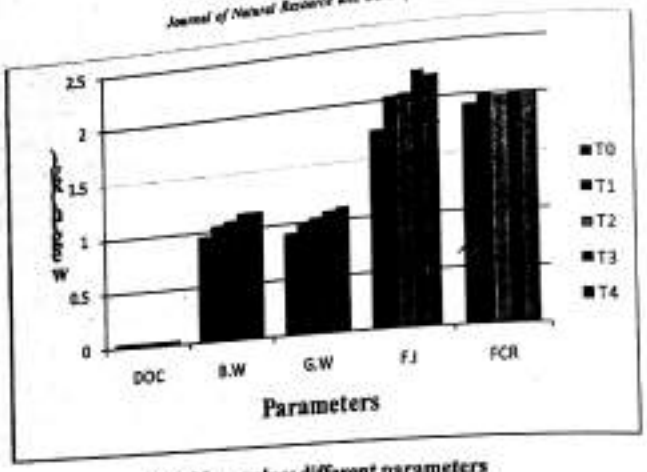

Fig 1 .Mean values different parameters

REFERENCES

Balog, B. D.; Kidd, W. E.; Huff, G. R.; Huff, N. C. and Rath, 2003. USDA/ARS/ PP& PSR, Fayetteville and N. B. Anthony, Department of Poultry Science, University of Arkansas (September2003) Effect of Cold Stress on Broilers Selected for Resistance or Susceptibility to Ascites Syndrome. *Poultry Science*82:1383-1387.

Beck, J.R.(1991).Zootechinica International, XIV(3): 30.

Gopalan, C; Ram, S. B. and Subramaniam, B. 1981 Nutritive value of Indian food. National Institute Nutrition, ICMR Hydrabad. India.

Nolan, J.; Hinch, G.; Twaites, J. and Walkden-Brown, S.(1999). Constrains to animal production, Chapter 2: Climati constrains, Lecturer Paper 12, *Animal Science Group* Publisher, Australia.

Ozkan, S.; Y. Akbas; Altan, A.; Altan, A.; Ayhan and Ozkan, K. 2003. The effects of short-term fasting on performance traits and rectal temperature of broilers during the summer season. Brit. *Poultry Sci.*, 44:88–95.

Sendecor ,C.W. and Cochran, W.G 1994 Statistical Method. Lowa State University Press, Ames.

Smith, M.O.; Sherman, L.L.; Miller, L.C. and K.R Robbins, 1995. Relative biological availability of manganese from manganese proteinate, manganese sulfate, and manganese oxide in broilers reared at elevated *temperatures* .*Poultry Sci.* 74:702-707.

Sobayo, R.A. 2005. Effect of ascorbic acid supplementation on the performance and haematological profile of pullets and layers. PhDthesis. Department of Animal Nutrition. Universityof Agriculture. Abeokuta. Nigeria. 214 pp.

Takahashi K.; Akiba Y. and Horiguchi, M. 1991. Effects of supplemental ascorbic acid on performance organ weight and plasma cholesterol concentration in broiler treated with propylthiouracil, In *British Poultry Science*32 (3):545-554.

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DIVERSITY OF FISH NEMATODE - *CONTRACAECUM INDICUS N. SP.* (NEMATODA : ASCARIDIDA, ANISAKIDAE) FROM MEERUT-DELHI REGION (INDIA)

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ABSTRACT

Nematodes constitute one of the most important groups of animals. Some of them are free-living in soil and water while others are parasites of plants and animals. The nematodes parasitizing the animals including man cause a number of diseases which may sometime results in the death of their hosts. One female specimen of the nematode belonging to the genus *Contracaecum* identified as *Contracaecum indicus n. sp.* was obtained from the intestine of fish collected from Meerut-Delhi region. The nematode posseases three lips without dentigerous ridges and well-developed interlabia while in *Contracaecum brevispiculum* interlabia was smaller.

Key words: Contracaecum indicus n. sp., fish intestine, Nematoda, Meerut-Delhi region.

Scientific advances over the past century have led to the improvement in lives of most of the peoples in both developed and developing worlds. But many of these benefits have not yet been produced in a sustainable way because the human population continues to grow and the diversity as well as abundance of many species diminishes. What happens to our world and to us and the creatures we share the world with in the future depends on the actions (Robert, 2002). Knowledge of parasite biodiversity contributes to new and exciting approaches to understand the structure, history and future of the fauna (Rudolphi, 1805; Baylis and Lane, 1920; Baylis and Daubney, 1922, 1923; Thwaite, 1927; D'Amelio *et al.*, 2012). Comparative baseline and archives intended for biodiversity are essential for recognizing the biotic responses of host-parasite system and the potential for emergence of disease across rapidly changing ecosystem (Leon-Regagnon, 2002). According to Scholz and Ditrich (1990), the body surface of an immature female *Gnathostoma spinigerum* found for the first time in the definitive host (*Felis catus f. domestica*) in Laos was studied using scanning electron microscope. All types of cuticular spines, which were one of the most important features for species identification of gnathostomid nematodes, together with their spatial arrangement. Diversity of species has precious relationship with virulence and hypersensitivity reactions in the host. The disease spectrum entirely depends on species and the emergence of any new species would bring new symptoms and evaluation of such parasites is necessary. Vertebrates are affected by both their external and internal environments. Internal environment, in turn, would be responsible for the necessary changes in the endoparasites. Brooks (2002) remarked that 21st century is to be considered a new age of discovery specially from biodiversity. One female nematode specimen of genus *Contracaecum* identified as *Contracaecum indicus n. sp.* was obtained from the intestine of fish of Meerut-Delhi region which has been described this communication.

MATERIALS AND METHODS

Fishes were collected from fish markets from Meerut-Delhi region (India). They were taken out from water and chloroformed. The alimentary canal was

cut open in the normal saline and the parasites were recovered from the intestine of fish. Parasites were transferred to normal saline (0.75% NaCl). After removing the saline with the help of a dropper, 70% alcohol was poured in the petridish to kill and fix the parasite in 90% alcohol with 2% glycerine. The preserved parasites were cleared in lactophenol for 15-24 hours and mounted in the same medium for enface-view study. The head of parasite was cut with a sharp blade and brought into desired position under the cover glass. All measurements are given in millimeters.

Scanning electron microscopic studies: After *in vitro* treatment the parasites were kept into the modified Kamovskys fluid separately according to the concentration of drug and exposure hours which were used as a fixative. The parasites were kept in the fluid at 4°C for 5-6 hours, thereafter they were transferred into 0.1 M cacodylate buffer solution then subsequent dehydration.

Dehydration: Absolute dry acetone was used as a dehydrating agent. Acetone was found to be advantageous as it was miscible with liquid carbon-di- oxide freon-13 used for critical point drying for dehydration. Acetone was prepared with different percentage as 30, 50, 70, 80, 90 and 95 and dehydration were carried out. After dehydration worms were subjected to critical point drying.

Critical point drying: The drying apparatus was properly installed and run with cold water circulation to cool the chamber about 20°C. The parasites were kept in loading baskets with dry acetone. They were removed in boats and placed into the frying apparatus. The inlet valves, connected to the CO_2 cylinders, were opened to fill the liquid gas rapidly. To avoid the back pressure the vent valves were opened.

The vent valves were slightly opened to maintain level of the liquid. The drain valves were opened to remove acetone. Flushing was carried out for 3-5 minutes. After flushing completed, the loading basket were filled with liquid CO_2 for impregnation in parasites. The steps were again repeated. The inlet valves were closed to allow the level of liquid CO_2 to fall to about the level of the top to the boat. The chambers were warmed by running warm water (36-38°C) and when the temperature was attained 32.5°C, CO_2 were evaporated and the drying was completed. The specimens were removed and mounted on the SEM stubs with double adhesive tape. After that, they were coated with gold approximately 350°A and the stubs were subjected to scanning electron microscopic studies and microphotography.

RESULTS AND DISCUSSION

Family: Anisakidae; Genus: *Contracaecum*, Species: *Contracaecum indicus n. sp.* Material One female, Host fish, Location intestine, Locality Meerut-Delhi region (India), Number of fish examine 27, Number of fish infected 13.

Type specimen: Halotype and paratype specimens deposited in the Department of Zoology, Meerut College (C.C.S. University), Meerut (India). (Fig. 1-4).

Coiled un-segmented worm with three lips without dentigerous ridge, head and mouth triangular. Mouth lead into chitinized bucal capsule. Oesophagus cylindrical and intestinal caecum extended anteriority. Vulva anteriority placed by two prominent upper and lower vulval flaps. The tail end contained short spine at tip (Fig. 3).

Female: The female measured 14.175 mm in length and 0.18 mm in width. Length of buccal capsule was 0.104 mm. Oesophagus measured 1.32 mm in length from anterior extremity. The width of anterior narrow oesophagus was 0.6 mm. The length of tail was 0.075 mm and contained a short spine at tip.

SEM studies: In scanning electron microscopic studies of *Contracaecum indicus n. sp.*, the anterior region showed finally striated cuticle depicting outer cortical region. The striations were very regular over the cuticle. Minute concricenisis and very minute protuberances present between the transverse

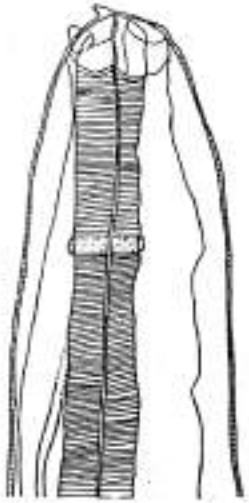

Fig. 1: Camera lucida diagram of *Contracaecum indicus n. sp.* female anterior region. x 150.

Fig. 2: SEM of *Contracaecum indicus n. sp.* female anterior region. x 500.

Fig. 3: Camera lucida diagram of *Contracaecum indicus n. sp.* female posterior region. x 150.

Fig. 4: SEM of *Contracaecum indicus n. sp.* female posterior region. x 1000.

Table 1: Comparison of *Contracaecum indicus n. sp.* with other species of the genus.

Parameters	*C. microcephalum* Rud (1809) Baylis (1920)		*C. circumvum* Rud (1819) Baylis and Daubney(1922)		*C. clavulatum* Schneider(1866)Baylis and Daubney(1923)		*C. contortum* Cobbold(1912) Baylis (1920)		*C. circumpapillatum* Gedoelst(1916) Baylis (1920)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Total Length	15-45	23-70	34.3-9.0	22-122	30-40	30-40	26	39.36	13.8	12.1-17.5
Total Width	0.9-1	11	0.35-0.175	0.75-3	1.1	2.1	0.6	0.3325	0.785	0.96-0.98
Length of oesophagus	2.8-3.6	2.8-3.6	3		1/15 of total length	1/13 of total length	3.9		3.8-3.9	3.8-3.9
Width of anterior narrow oesophagus										
Width of bulb									16-20	
Length of tail	0.23-0.21	0.44-0.5	0.2	0.36-1.7	0.2	0.5	0.22		0.14	0.35-0.44
Length of spicule: Small										
Length of spicule: Large	2.3-2.8		4.1-4.4		2.37		3.28		46	
Nerve ring from anterior end			0.55				0.5-0.57	0.5-0.57		
Length of tail process										
Length of buccal capsule					0.2	0.2				
Distance between striations										

striations. The protuberances and concricenisis were round in appearance. The anterior extremity showed three distinct lips with enclosing the triangular mouth. Each in the contained a pair of protuberant papillae each papillae consisted of a base and a triangular and a spine like fleshy papillae. The lip region also contained minute concricenisis and protuberances. The tail region also contained the cuticular transverse striations full of concricenisis and protuberances in the extreme posterior region. The striations appeared as concenaric rings of cuticle.

Railliet Henry (1912) reported key to the species of *Contracaecum* from fishes in South Asia. Members of new species of Genus *Contracaecum* is increasing day-by-day in different organisms (DÁmelio *et al.*, 2012). Detailed study conducted on the nematode found in the fish revealed that it belonged to genus *Contracaecum*. This nematode showed closeness with *C. brevispiculum* (Khan and Yaseen, 1969) but it differed from the latter in the presence of interlabia which was smaller in *C. brevispiculum* but in the present species interlabia was well-developed. The tail of present parasite contained short spine at the tips but in the *C. brevispiculum* tail was bluntly pointed. The striations were not present from anterior to posterior region in *C. brevispiculum* whereas *C. indicus n. sp.* striations were present and other reported new and old species interlabia, tail and striations were less developed in comparison to new species.

Female of *C. indicus n.sp.* measured 14.175 mm whereas females of *C. brevispiculum.* measured 12.75-17.14 (14.945) mm in length. Maximum thickness of the present parasite was 0.18 mm

whereas in *C. brevispiculum.* maximum width measured 0.14-0.48 (0.31) mm. Oesophagus of *C. brevispiculum* measured 0.73-0.82 (0.775) mm in length and 0.06-0.10 (0.08) mm in width whereas in present species it was 1.32 mm in length and 0.6 mm in width. Length of tail measured 0.13-0.15 (0.14) mm as in *C. brevispiculum.* whereas in *C. indicus n. sp.* it measured 0.075 mm. The nerve ring was situated at a distance of 0.16-0.18 (0.17) mm from the anterior extremity but in the present species 0.301 mm from the anterior extremity. Length of buccal capsule in *C. indicus n. sp.* was 0.104 mm. On the basis of these variations, the present species differed from all the reported species of genus *Contracaecum* new and old species and it revealed new diversity of species. Comparison of *Contracaecum indicus n. sp.* with different species of the genus is summarized Table 1.

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REFERENCES

Baylis, H.A. and Lane, C. (1920). A revision of the nematode family Gnathostomidae. *Proceedings of the Zoological Society*, London. 245-310.

Baylis, H.A. and Daubney, R. (1922). Report on the parasitic nematodes in the collection of the Zoological Survey of India. *Mem. Indian Mus.*, 7: 263-347.

Baylis, H.A. and Daubney, R. (1923). A further report on parasitic nematodes in the collection of the Zoological Survey of India. *Rec. Indian Mus.*, **25**: 551-578.

Brooks, D.R. (2002). Parasite systematics in the new discovery. In: *Tenth International Congress of Parasitology* (August 4-9, 2002). Vancouver, British Columbia, Canada. pp. 5-10.

D'Amelio, S; Cavellero, S; Dronen, N.O.; Barros, N.B. and Peggi, L (2012). Two new species of *Contracaecum* Railliet & Henry, 1912 (Nem,atoda: Anisakidaea)), *C. fagerholmi n. sp.* and *C. rudolphi F* from the bottom pelican, *Pelecanus occidentalis* in northern Gulf of Mexico. *Syst. Parasitol.*, **81**: 1-16.

Khan D. and Yaseen, T. (1969). Helminth parasites of fishes from East Pakistan. I. Nematodes. *Bull. Dep. Zool., Univ. Punjab (New Ser.)*, **4**: 1-33.

Leon-Reganin, V. (2002). Incorporating morphological and molecular data in biodiversity inventories. Parasites of Leopard Frogs. In: *Tenth International Congress of Parasitology* (August. 4-9, 2002). Vancouver, British Columbia, Canada. pp. 15-17.

Railliet, A. and Henry, A. (1912). Quelques ne´matodes parasites des reptiles. *Bull. Soc. Pathol. Exoth.*, *5*: 251-259.

Robert, M.M. (2002). *Current Science*. **82**: 169-173.

Rudolphi, C.A. (1809). Entozoorum sine vermium. *Intestinal. Hist. Natur.*, 2: 1-457.

Scholz, T. and Ditrich, O. (1990). Scanning electron microscopy of the cuticular armature of the nematode, *Gnathostoma spinigerum* Owen (1836) from cats in Laos. *J. Helminthol.*, **64**: 225-262.

Thwaite, J.N. (1927). On a collection of nematodes from Ceylon. *Ann. Tropical Med. Parasitol.*, **21**: 225-244.

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EFFECT OF DIFFERENT LEVELS OF IRON ON THE GROWTH PERFORMANCE OF BROILER CHICKS

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ABSTRACT

An experiment was conducted to evaluate the effect of different levels of iron*viz* 60, 80, 100 ,120on body weight, weight gain, feed intake and feed conversion ratio of broiler chicks. There were 4 treatments and the control without iron. Performance of broiler chicks evaluated on the basis of body weight, gain in weight, feed intake and feed conversion ratio indicated that there was no significant influence of treatments on the performance of broiler chicks based on these parameters.It was found that the supplementation of the diets with iron allowed a healthy, positive growth performance of the birds since there was no mortality.

Key words: Chick, level, iron.

Poultry chicken meat production plays a large part of food security in the face of the rapid increasing world population. It is critically important for this industry to continue to provide a great deal of a person's daily animal protein requirement. Growth of the broiler industry is characterized as robust but volatile (Anonymous, 2011). However, a major concern is the need to improve the quality of meat and self sufficiency. Consequently, the use of dietary alternatives, such as iron, has been recommended to enhance performance of broiler chicks. It is well known that trace minerals in basal diets affects growth performance, immune responses, and meat quality (Yang *et al*, 2011). Iron can correct or prevent anemia, in addition to being directly involved in hemoglobin functions (McNaughton and Day 1979). Iron deficiency anemia is a common nutritional deficiency and Ferrous fortification of diets is necessary to alleviate this problem.A deficiency of Fe may result from inadequate intake, for example, a high cereal diet low in animal protein or inadequate absorption, for example, gastrointestinal disturbances such as diarrhea or intestinal disease, as well as from excessive loss of blood (Anonymous, 2007). Therefore, an experiment was conducted to determine the effect of different levels iron on the growth performance of broilers chicks.

MATERIALS AND METHODS

Table 1: Treatments used in the experiment

Treatment	Level of supplements or the combinations
T_0	CONTROL (basal feed with no supplement)
T_1	60 mg iron
T_2	80 mg iron
T_3	100 mg iron
T_4	120 mg iron

Fifteen (15) day old broiler chicks of the same hatch were procured and reared in battery type cages. Each day old chick was weighed and distributed randomly among the 5 treatments. There were 3 chicks, referred to as 3 replications, per treatment. The production cycle was up to 5 weeks after hatching.Chicks were

fed with self-prepared standard broiler starter ration from day 1 to day 21 (3weeks) and then standard broiler finisher ration from day 22 to day 35 (4th and 5th weeks). The two types of ration (starter and finisher) were supplemented with different levels of iron (Table 1). The ration was fed *ad lib* to the birds.Data on body weight was recorded weekly to determine growth rate and weight gain of the chicks. To estimate the weekly feed consumption of the chicks, the weight of each feed bin was recorded weekly. The original weight of the bin was subtracted from the weight obtained each week to determine weight of feed consumed by the chicks. All broilers were offered with clean drinking water to be taken *ad lib* at all times.

RESULTS AND DISCUSSION

The mean body weight of day old chicks (DOC) for treatments T0 to T4 was42.67, 44.00, 46.00, 45.33, 48.00, , respectively. At the end of the production cycle, the mean body weight of five-week old chicks ranged from 1.07kg to 1.18 kg. The mean body weight (kg) of five-week old chicks for treatments T0 to T4 was1.07, 1.18, 1.15, 1.17 and 1.14, respectively. There were no significant differences (pd"0.05) among the treatments. The lowest mean body weight was 1.07 kg for treatment T0 and the highest was from T1 with 1.18 kg (Fig 1).

In terms of weight gain, the average weight gain of five-week old chicks ranged from 1.03 kg to 1.14 kg.The mean weight gain (kg) for each treatment from T0 to T4 was1.03, 1.14, 1.10, 1.13 and 1.09, respectively.There were no significant differences (pd"0.05) among the treatments. The lowest mean weight gain was 1.03 kg from T0 and the highest mean weight gain was 1.14 kg from T1 (Fig 1).

The average feed intake of five-week old chicks ranged from 1.91 kg to 2.03 kg. The mean feed intake (kg) for each treatment from T0 to T4 was 1.91, 1.95, 1.95, 2.03 and '2.01, respectively.There were no significant differences (pd"0.05) among the treatments. The lowest mean feed intake was 1.91 kg from T0, while the highest was 2.03 kg from T3 (Fig 1).

The feed conversation ratio of five-week old chicks had a mean range from 1.71 to 1.85. The average feed conversion ratio for each treatment from T0 to T4 was 1.85, 1.71, 1.77, 1.80 and 1.84, respectively. There were no significant differences (pd"0.05) among the treatments. The lowest mean feed conversion ratio was 1.71 from T1, while the highest was 1.85 from T0 (Fig 1).

McNaughton and Day (1978) concluded that iron requirements for hematological and growth responses are critically essential in broiler diets, while Oguz*et al* (2006) found that iron sulphate supplementation had a significant effect on live body

Table 1: Mean value of different parameters in different treatments

Treatments	Parameters				
	DOC (g)	Body Weight at 5th Week (kg)	Weight Gain at 5th Week (kg)	Feed intake for five weeks (kg)	FCR
T_0	42.67	1.07	1.03	1.91	1.85
T_1	44.00	1.18	1.14	1.95	1.71
T_2	46.00	1.15	1.10	1.95	1.77
T_3	45.33	1.17	1.13	2.03	1.80
T_4	48.00	1.14	1.09	2.01	1.84
Results	NS	NS	NS	NS	NS

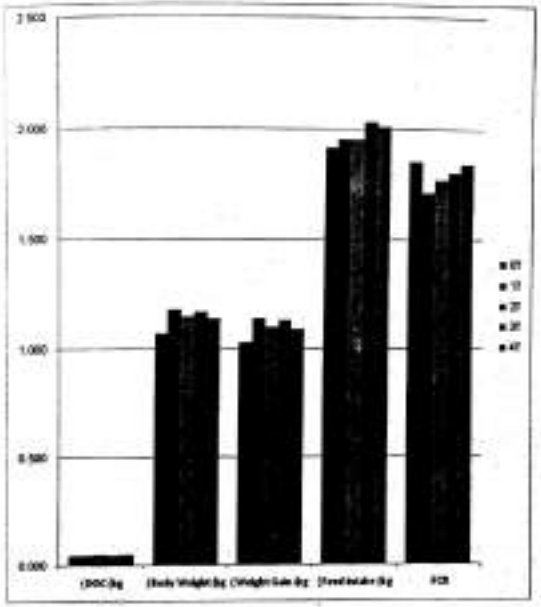

Figure 1 : Mean value of different parameters in different treatments

weights. However, Fairchild *et al* (2006) conducted trials to evaluate the effects of Fe concentration on broiler performance and found no differences in body weight, feed consumption, water consumption, mortality, or manure-soluble P in any of the trials.

In conclusion, the supplementation of the diets with iron allowed a healthy, positive growth performance of the birds, since there was no mortality. Ultimately, these trace elements could be considered as a good option to fortify broiler diets in order for farmers to produce healthy broilers with no or least mortality.

REFERENCES

Anonymous, (2011b). India poultry and Products Annual Report 2011.

Anonymous, (2007). Broiler Nutrition Specification. Ross 308: 1-7.

Fairchild, B. D.; Batal, A.B.; Ritz, C. W. and Vendrell, P.E. (2006). Effect of Drinking Water Iron Concentration on Broiler Performance. *J. Appl. Poult Res.* **15**: 511 – 517.

McNaughton James, L. and Elbert, J. Day (1979). Effect of Dietary Fe and Cu Ratios on Hematological and Growth Responses of Broiler Chickens. *J. Nutr.* **109**: 559-564.

OguzFatma, Karakas; Oguz, Mustafa Numan, HatipogluSefika and Gulay Mehmet Sukru (2006). The Effects of Iron Sulphate Supplementation of Diets Containing Cottonseed Meal on Performance and Haematological Parameters of Broilers. *J. Fac Vet Med UnivErciyes*.3(1): 9 – 14.

Yang, X. J.; Sun, X. X.; Li, C. Y.; Wu, X. H. and Yao, J.H. (2011). Effects of copper, iron, zinc, and manganese supplementation in a corn and soybean meal diet on the growth performance, meat quality, and immune responses of broiler chickens. *J. Appli. Poult. Res.* **20**: 263 – 271.

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DIVERSITY OF FISH NEMATODE - *PARANISAKIS LEVINI N. SP.* (NEMATODA: ENOPLIDA, ANISKIDAE) FROM MEERUT- DELHI REGION (INDIA)

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ABSTRACT

Nematodes constitute one of the most important group of animals. Some of them were free living in soil and water and other parasites were of plants and animals. The nematodes parasitizing the animals including man cause a number of disease which may some time results in the death of their hosts. In the present study, a new species of nematode obtained from the intestine of fish has been identified as *Paranisakis levini n. sp.* It possessed three prominent lips with small papillae detigerous ridges while in *P. sciaenae* dentigerous ridges was absent. The conical tail was provided with terminal spike contained lateral spine in the present species whereas *P. sciaenae* posterior region of female was unknown.

***Key words** : Paranisakis levini n.sp., Nematoda, fish intestine, Meerut-Delhi region.*

According to a World Health Organization (WHO) Expert Committee (1987) the overall impact of parasitic infection constitutes a significant health and social problem. Recent global estimate indicate that intestinal helminth infections were the most common infection in the world (Pawlowski, 1984). The number of nematode species in the world is uncertain (D'Amelio *et al*, 2012). According to Steiner (1960), about 9000 species have been described but these were doubtless only a fraction of those actually existing. Hyman (1951) thought that there must be at least 5,00,000 species in the world. Brooks (2002) emphasized that biodiversity assessment and emergence of bioinformatics have demonstrated that the biosphere is poorly known at all levels of organization. He further stated that the 21st century is to be considered a new age of discovery especially from biodiversity or diversity of species. One female specimen of the nematode belonging to genus *Paranisakis* identified as *Paranisakis levini n. sp.* was obtained from intestine of fish, the details of which have been described in this communication..

MATERIALS AND METHODS

Fishes were collected from fish market from the Meerut-Delhi region, India. They were taken out from water, chloroformed and the alimentary canal cut open in the normal saline. The parasites were recovered from the intestine of fish and transferred to normal saline (0.75% NaCl). After removing the saline with the help of a dropper, 70% alcohol was poured in the Petri dish to kill and the parasite post-fixed in 90% alcohol with 2 percent glycerine. They were cleared in lactophenol for 15-24 hours and mounted in the same medium for appropriate observations. Head of the parasites was cut with a sharp blade and brought into desired position under the cover glass and measured (in mm).

Scanning electron microscopic studies: After *in vitro* treatment, the parasites were kept into the modified Karnovskys fluid, separately according to the concentration of drug and exposure hours which were used as a fixative. The parasites were kept in modified Karnovskys fluid at 4°C for 5-6 hours after

that they were transferred into 0.1 M cacodylate buffer solution then subsequent dehydration.

Dehydration: Absolute dry acetone were used as a dehydrating agent. Acetone were observed to be advantage as it was miscible with liquid carbon dioxide freon-13 that were used for critical point drying for dehydration. Different concentrations of acetone (30, 50, 70, 80, 90 and 95%) was prepared and dehydration carried out in ascending steps. After dehydration, the worms were subjected to critical point drying.

Critical point drying: The drying apparatus was properly installed and run with cold water circulation to cool the chamber about 20°C. The parasites were kept in loading baskets with dry acetone. They were removed in boats and placed into the drying apparatus. The inlet valve connected to the CO_2 cylinders were opened to fill the liquid gas rapidly. To avoid the back pressure, the vent valve were opened.

The vent valves were slightly opened to maintain the level of the liquid and the drain valves opened to remove acetone. Flushing were carried out for 3 to 5 minutes. After flushing completed, the loading baskets were filled with liquid CO_2 for impregnation in parasites and the steps repeated again. The inlet valve was closed to allow the level of liquid CO_2 to fall to about the level of the top to the boat. The chamber was warmed by running warm water (36-38°C) and when the temperature attained 32.5°C, CO_2 was evaporated and the drying completed. The specimens were removed and mounted on the SEM stubs with double adhesive tape. Thereafter, they were coated with gold approximately 350°A and the stubs subjected to scanning electron microscopic studied and microphotography.

RESULTS AND DISCUSSION

Material: one female, Host: fish, Location: Intestine, Locality: Meerut-Delhi region, Number of fish examined: 26, Number of fish infected: 11, Family: Aniskidae; Genus: *Paranisakis*, Species: *Paranisakis levini n.sp.* Type specimen: Halotype and paratype, specimens deposited in the Department of Zoology, Meerut College (C.C.S. University), Meerut, India. (Fig. 1-4).

Elongated worm with three prominent lips each contained a small papillae dentigerous ridges. Mouth led into chitinized buccal capsule, oesophagus cylindrical and intestinal caecum extended anteriorly. Vulva anteriorly. The tail end was conical and tapered provided with terminal spike contained lateral spines (Fig. 3).

Female

The female measured 39.36 mm in length and 0.3325 mm in width. Length of buccal capsule was 0.62 mm. Oesophagus measured 6.45 mm in length from anterior extremity. The width of anterior narrow oesophagus was 0.185 mm. The length of tail was 0.3 mm.

SEM studies: The scanning electron microscopic studies of *Paranisakis levini n. sp.* the rim of the mouth contained indistinct large number of small papillae the cuticular striations were very clearly seen but the protuberances present were less in number. The tail region showed distinct cuticular striations. The striations appeared like concentric ring like structure. The cuticular protuberances were very distinct. The tip of the tail was contained a fleshy cuticular distinct caudal papillae.

The present nematode was showing closeness with *P. sciaenae* (Khan and Begum, 1971) but from the latter it differed in the presence of small papilla in *P. sciaenae* whereas *Paranisakis levini n. sp.* large papilla were found on the lips. Dentigerous ridges was present in *Paranisakis levini n. sp.* whereas in *P. sciaenae* dentigerous ridges was absent. The conical tail provided with terminal spike contained lateral spine in the present species whereas *P. sciaenae* posterior region of female was unknown. The nerve ring was distinct as in *Paranisakis levini n. sp.* situated at the distance of 0.62 from the anterior extremity whereas in *P. sciaenae* nerve ring was indistinct. Size of papillae, dentigerous ridge, tail, nerve ring differed in comparison to new species. Large papillae were found

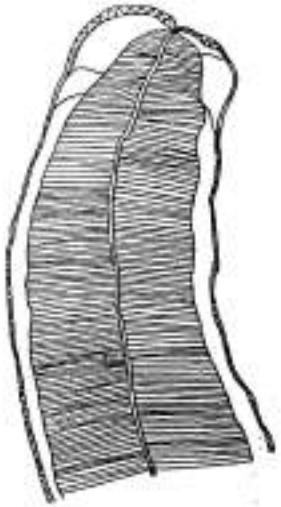

Fig. 1: Camera lucida diagram of *Paranisakis levini n. sp.* female anterior region. x 150.

Fig. 2: S.E.M. of *Paranisakis levini n.sp.* female anterior region. x 174.

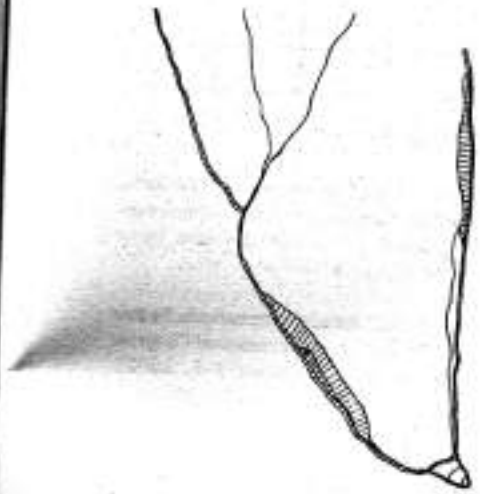

Fig. 3: Camera lucida diagram of *Paranisakis levini n. sp.* female posterior region. x 150.

Fig. 4: S.E.M. of *Paranisakis levini n.sp.* female posterior region. x 239.

Table 1: Comparison of *Paramisakis levini n. sp.* with other species of the genus.

Parameters		P. pastinacae Rud (1319) Baylis (1936)		P. sciaenae Khan and Begum (1971)		P. kherai Gupta and Garg (1976)		P. levinin sp.	
		Male	Female	Male	Female	Male	Female	Male	Female
Total Length		43.00	45-51	23.327			4.95-5.39		39.36
Total Width		0.37	0.33-1.2	0.312			0.15-0.16		0.3325
Length of oesophagus		1/13 of total length	0.103				0.65-0.76		6.45
Width of anterior narrow oesophagus				0.13					0.135
Width of bulb									
Length of tail			0.53-0.73	0.103			0.032-0.038		0.3
Length of spicule	Small	0.53							
	Large								
Nerve ring from anterior end							0.10-0.14		0.62
Length of tail process									
Length of buccal capsule									0.09
Distance between striations									

in the present species whereas in *P. sciaenae* it was smaller. On the basis of above variations present species differed from all the reported species of the genus *Paramisakis* new and old species and it revealed new diversity of species. Comparison of *Paramisakis levini n. sp.* with different species of genus *Paramisakis* is given in Table 1.

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REFERENCES

Baylis, H.A. (1936). *The Fauna of British India, including Ceylon and Burma. Nematoda. Vol. I. Ascaroidea and Strongyloidea.* Taylor & Francis, London. 408 p.

Brooks, D.R. (2002). Parasite systematics in the new discovery. In: *Tenth International Congress of Parasitology* (August 4-9, 2002). Vancouver, British Columbia, Canada. pp. 5-10.

D'Amelio, S; Cavellero, S; Dronen, N.O.; Barros, N.B. and Peggi, L (2012). Two new species of *Contracaecum* Railliet & Henry, 1912 (Nematoda: Anisakidaea)), *C. fagerholmi n. sp.* and *C. rudolphi F* from the bottom pelican, *Pelecanus occidentalis* in northern Gulf of Mexico. *Syst. Pasrsitol.*, **81**: 1-16.

Gupta, N.K. and Garg, V.K. (1976). On taxonimical and histochemical observations on a new nematode of the genus *Paransakis*. Baylis (1923). *Rivista Parasitol.*, **37**: 265-275.

Khan, D. and Begum, A. (1971). Helminth parasites of fishes from West Pakistan. I. Nematodes. *Bull. Dep. Zool. Univ. Punjab (New Ser.)*, **5**: 1-22.

Steiner, G. (1960). In: *Nematology* (Sasser, J.N. and Jenkins, W.R. eds.). North Carolina Univ., Carolina Press, Chapel Hill. pp. 3-7.

WHO (1987). *Prevention and Control of Intestinal Parasitic Infections.* Report of a World Health Organization Expert Committee, Technical Report Series. WHO, Geneva.

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EFFECT OF METABOLIC SIZE OF COWS ON BACTERIAL QUALITY OF THEIR MILK

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ABSTRACT

The present study was undertaken to determine standard plate count (SPC) for total bacteria, lactic acid bacterial count (LABC), lipolytic bacterial count (LBC), Proteolytic bacterial count (PBC) and coliforms for determining bacterial quality of raw milk as influenced by metabolic size of healthy crossbred cows by dry full hand method of milking at SHIATS dairy farm Allahabad. The analysis of variance showed significant effect of metabolic size on SPC, LBC, and coliforms but showed no significant differences in LABC and PBC in raw milk. The result indicated that the overall rating of bacterial quality was found better for raw milk produced by 262 to 296, and 297 to 333 followed by cows of 190 to 225 and 226 to 261 metabolic size. If the quality is judged on the basis of coliform than the quality of milk of was better from cows of metabolic size 226 to 261 and 297 to 333 followed by cows of 262 to 296 and 190 to 225 metabolic size.

***Keywords** : Crossbred cows, bacterial quality, metabolic size.*

India is an agriculture country and livestock sector in an integral part of agriculture. It is the back-bone of India's economy in the form of income, employment and foreign exchange earning. It is estimated that dairying sector alone contributes 15% of the Gross National income (Raju, 2001).Livestock sector accounts for about one third share of agriculture and allied sector to national GDP, thus emerging as an important subsector of agriculture. Milk is a clean lacteal secretion obtained milking of healthy milch animal, properly fed and kept excluding that obtained within 15 days before and 5 days after calving. Spoilage of milk due to bacterial action is estimated to be 10 percent of the total milk production in India (Chakraborti et al., 1986). In terms of quality of milk and productivity per dairy animal, India ranks bottom among the major dairy nations. In comparison to average bacterial count of raw milk 10^5 per ml in most of the European countries and USA, in India it ranges from $5x\ 10^5$ to 10^8 per ml. Therefore milking of animals should be done hygienically to preserve its freshness for longer time. Production of clean and safe milk should be fundamental objectives of all dairy farmers. Fresh raw milk contains micro-organism and the density of micro-flora largely depends upon the health of cow, sanitation of hind quarters and udder, cleanliness of container etc. Clean milk is the one which is not only without visible dirt but also in broad sense used to denote raw milk obtained from healthy animal produced and handled udder hygienic condition and have only small number of harmless bacteria. Thus the size of animal body is likely to contribute to the density of contaminations in raw milk which might be proportional to the metabolic size of animal body.

MATERIALS AND METHODS

An experiment was conducted in herd of SHIATS dairy farm consisted of crossbred cows (crosses of Jersy Brownswiss and Holstein) and determine the SPC, lactic acid bacterial count, lipolytic

bacterial count, proteolytic bacterial count and coliform in raw milk. Metabolic size of cows of SHIATS dairy farm was determined by standard formula Metabolic size = Body weight x 0.75 because size of an animal is proportional to its metabolic rate (Prasad and Neeraj, 2009). Only 12 healthy cows free from mastitis and other noticeable injuries were selected and divided into 4 groups of three cows in each for four treatments of metabolic size viz. 190 to 225 (M_1), 226 to 261 (M_2), 262 to 296 (M_3), 297 to 333 (M_4). All experimental cows were housed in a tail to tail barn and managed under similar management conditions. Sanitary precautions like clipping of long hair on the under and flank, grooming washing of hind quarter, wiping udder with towel soaked in 2% Dettol solution, tying tail with legs etc, were taken care. Cow was milked by dry full hand method of milking. Two streams of fore milk from each quarter of udder were discarded. A representative sample of 200 ml milk was collected from each cow directly into sterilized conical flasks and plugs replaced immediately. Milk samples were brought to laboratory for determination of bacteria namely standard plate count (SPC), lactic acid bacterial count (LABC), lipolytic bacterial count (PBC) and Coliforms per ml. The data on compositional ingredients were tabulated and subjected to analysis of variance techniques (ANOVA) as per randomized block design (RBD) of Snedecar and Cochran (1994) to determine influence of metabolic size on different bacterial parameters of raw milk.

RESULTS AND DISCUSSION

The mean SPC (10^4) per ml observed was 260.50 in milk of cow of M_2 followed by 259.67 in milk of cow of M_1, 235.50 milk of cows of M_4 and 222.33 in milk of cows of M_3. The differences in these values were significant. The mean LABC (10^3) per ml milk was recorded as 35.25 in milk of cows M_2 of followed by 27.0 in milk of cows of M_4, 26.66 in milk cow of M_1 and 23.33 in milk of cow of M_3. The differences in these values of LABC were non-significant. The mean LBC (10^2) per ml was 16.75 observed in milk of cows of M_2 followed by 15.66 in milk of cows M_3, 12.33 in milk of cows of M_1 and 10.0 in milk of cow M_4. The difference in these Value of LBC were Significant. The mean PBC (10^2) per ml was 21.3 M_1 followed by 20.0 in milk of cows of M_4, 19.5 in milk of cows of M_2 and 18.66 in milk of cows of M_3 of metabolic size The differences in these value of PBC were non Significant.

The mean Coliform per ml was 1.67 recorded in milk of cows of M_1 followed by 1.15 in milk of M_3, 0.50 in milk of cows M_2 and 0.50 in milk of cows of M_4 metabolic size. The differences in these values of coliform were significant.

Table1 : Mean Values of parameters according to metabolic size (M) of cows

Bacteria/ml	M_1 (190-225)	M_2 (226-261)	M_3 (262-296)	M_4 (297-333)	
SPC(10^4)/ml	259.67^a	260.50^{ab}	222.33^b	235.50^b	S (30.632 CD)
LABC(10^3)/ml	26.67^a	35.25^a	23.33^a	27.00^a	NS
LBC(10^2)/ml	12.33^a	16.75^{ab}	15.66^{bc}	10.0^c	S (3.474 CD)
PBC(10^2)/ml	21.30^a	19.50^a	18.66^a	20.0^a	NS
Co:Eforms/ml	1.67^a	0.50^b	1.15^{ab}	0.50^b	S (0.869 CD)

CONCLUSION

It was concluded that the bacteriological quality of raw milk adjudged on the basis of standard plate bacterial count was better in cows of metabolic size 262 to 296, and 297 to 333 followed by cows of 190 to 225 and 226 to 261 metabolic size. If the quality is judged on the basis of coliform than the quality of milk of was better from cows of metabolic size 226 to 261 and 297 to 333 followed by cows of 262 to 296 and 190 to 225 metabolic size.

REFRENCESS

Chakraborti, B.K.; Chaudhary, S.S.; Alex, K.A.; Jacob, G. and Soni, G.J. 1986.Application of the lactoperoxidase system for preserving buffalo milk production in Indian village. Milchwissenschalt, 41:16.

Prasad J. and Neeraj, 2009 Animal husbandry and Dairy Science, Kalyani Publications, P-3.

Raju, DT. 2001 Opinion of the farmers rearing crossbred cows. *The veterinarian*, 25 (10):7-10

Snedecar, G.W. and Cochran, W.G . 1994 Statistical method 8th Ed. The Lawa Stat University Press Ames Lowa USA.