PREPARATION OF SUPPLEMENTARY FOOD WITH EXTRACTED PROTEIN FROM A CHEAP SOURCE

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ABSTRACT

Khesari (*Lathyrus sativus*) is the cheapest pulse in Bangladesh. It constitutes about 30% of total pulse production in the country. It has a high protein content, about 28-30% compared to 20 -22% in other pulse. However when the pulse is eaten in excess amount (>60 % of total calorie) for a long time (2- 3 months) a debilitating paralytic disease of the lower limbs, lathyrism, shows up in some individuals. The disease is caused by a neurotoxin ß-N-oxalyl-α, ß-diamino propionic acid (ODAP) present in the ripe Khesari seed as a non-protein amino acid analogue.

To circumvent the toxin problem and to make the best use of Khesari protein, protein was extracted from the pulse by a simple 2-step procedure and purified to a concentrate (*Lathyrus* protein concentrate, LPC), which was virtually free from the toxin ODAP. The present study was done to investigate the chemical characteristics of the LPC, and to prepare a supplementary food as a growth promoter.

The protein recovery from Khesari was 66% . The content of the toxin in LPC was 0.0012% only, as against 0.806% in the seed (i.e. virtually free of the toxin). The *in vitro* digestibility of LPC was 99%, compared to 99.9% for casein. The protein fractions belong to the globulin family of molecular weight 113,000, 100,000, 55,000 and 24,000 respectively as is found in other pulses. As expected LPC had rather low quantities of methionine and cysteine but had moderately high quantity of lysine. All other amino acids were present in appreciable quantities.

The nutritive value of the LPC was estimated in growing rats by determining its PER (Protein Efficiency Ratio), BV (Biological Value) and NPU (Net Protein Utilization). Growing rats (body weight 126 to 158g in one experiment and 66 to

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76g in another experiment) were divided into five groups each receiving one of the following diets: (i) Non-protein diet, (ii) Skim-milk protein diet, (iii) LPC diet, (iv) Rice protein diet and v) Rice protein+LPC diet. As can be seen, diet v was the food supplemented with LPC.

It was observed that LPC alone showed a rather poor growth promotion ability but when it was mixed with rice (rice + LPC) the ability increased to almost that of skim-milk in respect of PER, BV and NPU. (A recent human feeding experiment supports the above findings in rats).

LPC also showed some influence on lipid metabolism. Blood cholesterol level was lower in rats receiving LPC than those receiving skim-milk protein.

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LIST OF ABBREVIATIONS

α	=	Alpha
App.	=	Approximately
ß	=	Beta
BOAA	=	Beta-N-Oxalyl-L-alanine
°C	=	Degree celsius
et al.	=	and associates
g	=	Gram
h/hr	=	hour
L	=	Litre
LPC	=	*Lathyrus* Protein Concentrate
m.w.	=	molecular weight
µg	=	Microgram
µl	=	microlitre
ml	=	Millilitre
min	=	Minutes
nm	=	nanometre
N	=	Normal concentration
%	=	Percent
ODAP	=	B-N-Oxalyl-L-α, ß-diamino propionic acid
PAGE	=	Polyacrylamide Gel Electrophoresis
pH	=	-Log of hydrogen ion concentration
r.p.m	=	rotation per minute
SDA	=	Sodium Dodecyl Sulphate
v/v	=	volume by volume
w/v	=	weight by volume
w/w	=	weight by weight

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Chapter 1

INTRODUCTION

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1. INTRODUCTION

Protein undernutrition is a commonplace for millions of the people of the poor Third World countries. The children of growing age are the worst sufferers. They suffer from kwashiorkor and other protein related diseases. The adults also suffer from protein undernutrition, resulting in low body vitality and mental competence. Severely affected pregnant mothers produce low birth weight babies who often do not survive the hostile infectious environment in which they are born and brought up.

The problem of protein malnutrition among infants and young children in Bangladesh is well recognized. This is due to the fact that the animal protein foods are scarce and very expensive and the people of the low and medium income groups cannot get enough of them to meet their protein requirements (UNROD, 1973). Emphasis has been given to combat protein malnutrition by using more plentiful and cheaper plant proteins (Bressani and Elias, 1968).

Among the plant sources grown in Bangladesh, pulses contain considerable amount of proteins. Legumes are nutritionally important foods because of their relatively high protein content (Aykroyd *et al.*, 1964). Pulses have traditionally been consumed mostly as cooked dahl to supplement the staple food but the infants and young children have problems with pulses due to the presence in them of some indigestible carbohydrates (Aykroyd *et al.*, 1964).

Pulses and beans are rich in the essential amino acid lysine (Roy *et al.*, 1989) and therefore when mixed with cereals which are deficient in this amino acid, form by mutual supplementation a good quality protein, the so called poor man's protein (Davidson *et al.*, 1979).

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Khesari (*Lathyrus sativus*) is potentially an extremely valuable pulse crop, which is drought resistant, protein rich and cultivated extensively in almost all areas of Asia and Africa. It is generally grown under diverse ecological condition (Gutierrez and Vences, 1995).

Khesari, also called Grasspea, is cultivated in Bangladesh since time immemorial (Sarwar *et al.*, 1995). It accounts for as much as 30% of the total pulses produced in Bangladesh, (Kaul *et al.*, 1989). *Khesari* is not only cheap, it is also nutritious. The seed has 27-28% protein as against only 18.0% in *Cicer arietinum* (Chana or chhola) and provides 354 Kcal per 100g (Roy *et al.*, 1989). It has been analyzed that price of 10g protein from Khesari is the cheapest of all other vegetable and animal sources (Table 1).

Table 1: Prices of 10g protein of different food items.*

Food Items	Price of 1 Kg (Taka)	Tk. per 10g protein
Rice	15	2.14
Wheat	14	1.27
Milk (Powder)	200	6.70
Egg	60	4.61
Fish	100	7.14
Beef	70	3.18
Lentil	40	1.6
Khesari	20	0.68

* According to current market price.

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1.1 Origin and distribution *Lathyrus sativus*

Lathyrus sativus is indigenous in southern Europe and Western Asia. It has spread as a weed and also as a crop. It has been extensively cultivated in the Indian sub-continent (India, Bangladesh, Myanmar, Nepal and Pakistan), Iran and to a small extent in the middle Eastern countries, Southern Europe, and parts of Africa and South America Westphal 1974).

1.2 Climate and soil

Lathyrus is a temperate crop. It is cultivated in the cold winter months in the Indian sub-continent. It can grow well under moderate temperature ranging from 10-30°C (Kay, 1979). *Lathyrus* is the hardiest of the pulse crops because it can tolerate flooding and droughts. It can be grown in areas of low rainfall (300 - 500 mm) and also in areas of high rain fall (up to 1500 mm) such as in Bangladesh. This tolerance to extremities of flooding and drought has made it very popular in drought prone areas where heavy rain may occur for brief periods (Pulses in Bangladesh, 1982).

Lathyrus can be cultivated over many types of soils ranging from very poor marginal soils to rich black cotton soils. Most commonly, *Lathyrus* is cultivated as a second season crop in low lying rice fields in clay soils which remain wet for a long time (Nezam uddin, 1970). It can withstand short drought periods and moderate soil salinity, better than peas (Serov, 1974).

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1.3 Agronomy

1.3.1 Seed bed preparation

Lathyrus is one crop that requires only minimum tillage. As it is usually sown after a rice crop, when the seed is still wet, no tillage is possible. In uplands (medium-high lands) one to two ploughing may be given depending on farmers choice (Pulses in Bangladesh, 1982).

1.3.2 Source of seed

The seeds of *Lathyrus* are hardy and can be stored for more than 2 years without appreciable loss in germinability and vigour. Kotlyarov (1975) reported that seeds obtained from the lower nodes had high seed weight and higher germination seeds germinate best at an optimum temperature of 20°C. Higher temperature inhibits germination (Agrawal *et al.*, 1980).

1.3.3 Time of planting

Planting time for *Lathyrus* is from the end of October to early December. The planting time depends on the harvest of the preceding rice crop. If planting follows Aus or Jute, then sowing can be done in October. No studies are reported regarding optimum sowing time (Pulses in Bangladesh, 1982).

1.3.4 Fertilizer response and recommendation

Research conducted at JNKVV, Jabalpur, India, has indicated that *Lathyrus* responds to up to 20 kg N and 40 kg P_2O_5 (Anonymous, 1972). However, considering the cost of fertilizers and farmers' reluctance to use them, Singh

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(1975) recommended 10 kg N and 20 kg P_2O_5 to be applied at the time of planting. No fertilizers, not even FYM, are given to *Lathyrus* in Bangladesh.

1.3.5 Nodulation and nitrogen fixation

Being a legume, *Lathyrus* forms nodules with *Rhizobium leguminosarum* and fixes nitrogen symbiotically. Kolotilace (1976) indicated that seed inoculation with effective *Rhizobium* strain yielded similarly to those supplied with N.P.K. Bhuiya *et al.* (1982) studied the performance of locally isolated Bangladeshi strains of *Lathyrus rhizobium*. In a net house experiment, they (1982) studied variability for nodule formation on main and lateral roots. In the field study (1982) variability was observed for nodule number, dry matter and N uptake by the plant. A few strains were found to be consistently superior in performance with regards to degree and size of nodules set.

1.3.6 Response to irrigation

Traditionally, *Lathyrus* is not irrigated. In a study in USSR, Mozheiko *et al.* (1975) used sewage water to irrigate *Lathyrus*. Irrigation to maintain soil moisture contents above 80% of field capacity from time of emergence to maturity gave highest seed yield of 3.12 t/ha and fresh fodder yield of 34.5 t/ha and 7.7 t/ha of hay. Sewage irrigation tended to reduce the seed weight but increased the N.P.K. content and nutritive value of fodder and hay.

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1.4 Physiology

1.4.1 Flowering and podding

Lathyrus needs long days for early flowering. Miroshnichenko and Kolotilov (1976) reported that *Lathyrus* flowered in 40-47 days at 40°-50° latitude.

Generally, the flower color in Khesari is highly correlated with the seed colour the blue, pink, or red clor flowers usually produce speckled, coloured seeds (Campbell and Deshpande, 1995). From the study of Sarwar *et al.* (1995), it was showed that drought proned seed size was significantly lower than that of from coastal area. The distribution of drought proned seed was skewed to the right, on the otherhand that of saline prone seed was skewed to the left. The skewness of both the populations were significant.

Sarwar *et al.* (1995) also showed that the days to first and 50% flowering were higher in drought prone Khesari than that in saline prone. The distribution of both the population were significantly skewed to the left for both the traits. In early flowering lines vegetative and reproductive phases continued simultaneously. While late flowering type started flowering after completion of certain stage of vegetative phase, it continued to grow simultaneously with reproductive phase. The later type is busy in nature and could be suitable for fodder purpose.

Sarwar *et al.* (1995) also showed that blue flower was the only flower colour in *Lathyrus* populations in Bangladesh and it is the predominant color. A lot of flower colour was identified such as white, pink, deep pinck, red, light violet, reddish blue, pinkish blue and light blue and all these flower colour mutants rose from blue color. So it was hypothesized that flower colour in *Lathyrus sativus* is governed by multiple alleles.

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Flowering was delayed by 0 to 4, 4 to 15 and 7 to 19 days when day lengths were 14, 12 and 10 hrs respectively. Short day lengths tended to decrease plant height, dry matter production and the size of leaves of the plant. Pods formed near the base of the plant had less seed abortion than those on the middle and upper nodes (Miroshirichenko and Kolotilov, 1976).

1.4.2 Pests

Aphids, *Aphis craccivora* are reported to be a major pest in India and Bangladesh. Singh (1975) reported that an almost total crop loss can occur with severe infestation. Heavy infestation of aphids was noticed on *Lathyrus* in Ishurdi areas in 1981-82.

1.5 Harvest and postharvest practices

The leaves turn yellow and pods turn grey when mature. The mature plants are normally pulled out or cut with a sickle near the base. The harvesting time is March to April.The plants are then stacked and allowed to dry in the field on the threshing floor for 7-8 days. The plants are spread out on the threshing floor and beaten with stick. The seed is then winnowed and cleaned. Drying of seed for 1-2 days may be necessary before it is stored. Most of *Lathyrus* harvest is stored for domestic use, only small part is sold. The seed can be stored without difficulty in ways similar to other pulse crops (Pulses in Bangladesh, 1982).

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1.6 Nutritive value

Seeds of several legume species of the genus *Lathyrus* are produced extensively in Bangladesh and India, some countries of North Africa and also in Southern Europe. The seeds are rich in protein (23 to 28%) with adequate concentration of most inorganic elements and amino acids particularly lysine but deficient in methionine and cysteine (Chowdhury, 1988; Low *et al.*, 1990; Rotter *et al.*, 1991; Yusuf *et al.*, 1995). It also contained iron and B vitamins (Shobhana *et al.*,1976).

Lathyrus sativus (Khesari) seed has 27-28% protein as against only 18% in *Cicer arietinum* (chana or chnola) and provides 354 Kcal per 100 g of seed (Ludolph *et al.*, 1987). Sen *et al.* (1978) found 29.5% crude protein, 7.8% crude fibre and 5.4% total ash as against 24.8% protein in lentil and 20% protein in chickpea. Fermented sample of *Lathyrus* seed showed higher protein content than the control sample but lipid content decreased, Ash content increased almost 30% after fermentation. In general the level of dietary fibre decreased in fermented seed meal, total dietary fibres decreased by 30%, hemicellulose decreased by 47% and lignin content decreased by 43%. These decreases may be beneficial especially for consumers of high amounts of *Lathyrus.* After fermentation the saturated fatty acid palmitic acid and unsaturated oleic acid increased while the polyunsaturated fatty acids, linoleic and linolenic decreased significantly (Lambein *et al.*, 1995).

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1.7 Uses

Khesari serves a dual purpose the grain is used for human consumption and the straw is used as an animal feed.

The grain is used as a complementary or sole source of calories and protein, mostly by the poor and landless labourers. The grain can be boiled whole but most often they are processed through a `dal' mill to obtain split dal. Dal is the most common item sold in the retail markets. The flour from *Lathyrus* seed is also sold in the market as basan. In many parts of Bangladesh roti (leavened bread) made out of Khesari is a staple for landless labourers during the lean periods (Gowda *et al.,* 1982).

Lathyrus is also very tasty and possesses an appetizing flavour. It is usually consumed as a vegetable soup and such consumption is often limitted to the poorer section of the population. However, concentrated preparation of the dal are also occasionally consumed in affluent families as a change of taste (Naved *et al.,* 1990).

In Ethiopia, several food items are prepared with Khesari known as grasspea including hot curry (shiro-wot), roasted grain (Kolo), boiled grain (Nifro) and dry bread or kita (Alelign and Regassa, 1989).

The survey report of Amruth and Bhat, (1995) showhed that the people of South India area continue to consume *Lathyrus sativus* but in reduced quantities for varying periods ranging from once in a month to daily, at least during post harvest season.

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Kitta is eaten mainly at times of acute food shortage (Tekle-Haimanot et al., 1993). Khesari is also an excellent fodder for cattles in our country (Naved *et al.* 1990) In studies at BARI, Joydepur, fodder yields of 7-10 t/ha were obtained in inter cropping with maize without affecting grain yields of maize (Gowda *et al.*, 1982). These legume seeds are cheap and should be considered in the formulation of poultry diets particularly at a time when scarcity and high cost of animal protein feeds stuffs have been major constraints to poultry producers (Chowdhury, 1995). Although lathyrogens from certain species of *Lathyrus* showed positive toxic effect on poultry it is possible to derive nutritional benefits from feeding seeds if used at dietary levels that have been proved to be safe for production purposes.

1.8 Antinutritional factors

Lathyrus is an ideal sustainable agriculture crop which will provide both human and animal food. Its major disadvantage is the presence of the neurotoxin B-N-oxalyl-L-α, ß-diamino propionic acid, ODAP (or BOAA) which can cause toxicity in animals and humans when excessive quantities are consumed. (Murti *et al.*, 1964). New lines of *Lathyrus* are being developed to maximize the agronomic potential and reduce or eliminate the toxic component (Briggs *et al.*, 1995).

1.9 Lathyrism : A crippling problem

The consumption of Khesari increases amongst the poor during the lean season of the year when rice and other cereals become too expensive (Haque *et al.*, 1989). This happens during the months of August to October in Bangladesh. The situation is aggravated during famines when cereals become scarce and the buying capacity of the poor comes down to the minimum level, Khesari becomes the staple food, in many cases the only food as an alternative to starvation during

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those crucial months. And then, the disease, lathyrism, strikes (Naved *et al.*, 1990).

Lathyrism, a debilitating neurological disorder caused by excessive prolonged intake of *Lathyrus sativus*, is thus known to man for centuries, several outbreaks of lathyrism were recorded in Europe (including Russia), Asia, and Africa during the 17th and 18th centuries (Kojewin Koff *et al.*, 1989; Stelye, 1957). In India also, lathyrism is known from ancient times, the first authentic description of the disease, however, being that given in 1844 by General Sleeman (Sleeman, 1884). Today, while the disease has been eradicated from Europe, it is still crippling thousands of people in some developing countries of Asia and Africa, particularly India, Bangladesh and Ethiopia (Dwivedi, 1989; Yusuf and Lambein, 1995).

In Bangladesh the last epidemic of lathyrism was experienced during the food scarcities of 1972-74, (Mannan, 1985; Aall, 1976; Ahmad *et al.*, 1981). After 1972-74, because of increased awareness about the toxic effect of *Lathyrus* and because no serious food scarcity like that in 1973-74 had occurred, the prevalence of lathyrism decreased in the country but new cases are still reported from the areas at risk (Shaw Unnayan, 1988) and the fear of an outbreak cannot be ruled out in the event of drought or flood not tackled properly.

From the study of Pandey and Kashyap (1995) it was found that in South-Eastern part of Madhya Pradesh, India where *Lathyrus* is not produced in large quantities, very few cases of human lathyrism were known. On the contrary in Rewa division, the production of khesari was lower but a high incidence of human lathyrism was reported from there. The cause of the high incidence has not yet been explained but might be due to dietary habit or to poor economic condition in the Rewa division.

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As mentioned earlier, lathyrism occurs in man when the *Lathyrus* seed is consumed in large excesses (e.g. 60% or more of total mass of food consumed) for several months. The attack usually comes suddenly; a man goes to bed in good health but cannot get up from bed in the morning, or a farmer ploughing in the field suddenly falls on the ground due to numbness of the legs. Lathyrism is characterized by signs of muscular rigidity and weakness of the leg muscles. In mild cases there is a slight bending of the knees and difficulty in running and the patient walks with bent knees (spastic paraplegia). As the disease progresses the patient walks with crossed gaiting with a tendency to walk on the toes (spastic parapresis). At this stage the patent is forced to use one or two sticks for support (Roy, 1988). Complete paralysis of the legs may develop in extreme cases (Ganapathy, 1988; Ludolph, 1987).

A long search for the toxic principle of *Lathyrus* seed finally led to the discovery of beta-N-oxalyl-L-alanine (BOAA) (also called beta-N-oxalyl-L-alpha, beta-diamino propionic acid, ODAP), a neuroexcitatory non-protein amino acid, as the lathyrogenic agent in animals (Rao *et al.*, 1964; 1967 Murti *et al.*, 1964).

Subsequently, a primate model for human lathyrism was developed by feeding *Lathyrus sativus*. (Ross *et al.*, 1985) and by administration of BOAA (Spencer *et al.*, 1984, 1986).

1.10 Removal of toxin from *Lathyrus sativus*

1.10.1 Physico-chemical detoxifiction

Various methods were tested for the removal of *Lathyrus* toxin. These included the age old procedure of boiling/steeping in water for verification. After having

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done a large number of tests under various conditions, one single method was finally found to be the best, easiest and the simplest (see later).

Steeping khesari seed for 1 hr with water was a very effective method for removing the *Lathyrus* toxin; the removal being 77.7%. This water steeping method was suggested many years ago by Mohan *et al.* (1966) in India. The saturated lime water method of Jahan and Ahmad (1984) although very effective (90-100% toxin destroyed), makes the seed extremely limy and distasteful. Even ¼th saturated lime water, that results in less than 50% toxin removal makes the seeds limy (Naved *et al.*,1990).

Detoxification of *Lathyrus sativus* seeds by steeping in excess water (Tekle Haimanot *et al.*, 1993) by autoclaving (Rotler *et al.*, 1990) or by washing, steaming and fermenting with 0.05% tempeh inoculum (Mosleuddin and Hang, 1987) has been reproted without sufficiently satisfying effects on removal of ODAP or without concurrent loss of essential nutrients like vitmains.

So far the best method developed for detoxification of Khesari is the steeping method with sodium bicarbonate. With steeping in 1.0% $NaHCO_3$ solution for just 20 min, 70% of the toxin was found to be removed/destroyed and this treatment does not disturb the taste of the seed. However, the method as any other steeping methods, has the inherent disadvantages that it involves cost of heating (up to boiling water) which may be to expensive for many households (Naved *et al.*, 1990). Also, steeping for 20 min and then baking sometimes might be too long for the hungry farmer to wait after the whole days of work (Dwivedi, 1983).

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Direct backing of Khesari paste with sodium bicarbonate overcomes these problems and the results were quite encouraging as much as 50% of the toxin was removed when chapati is made with 1.86g $NaHCO_3$ per 100g seed. In this method, no extra heat and extra time are needed. Also water is not drained out, so no nutrients are lost, moreover, the chapati taste normal when prepared the above way (Naved *et al.*, 1990).

However the concentration of $NaHCO_3$ used (1.86 g/100g seed) is higher than the maximum recommended salt content of diets namely 1.1%. This problem may be easily solved by diluting the seed paste say 50:50 W/W, with a cereal, e.g. potato, wheat, or even rice if available. That would bring down the salt ($NaHCO_3$) concentration to 0.93% (lower than the thereshold level) and also the toxin level to only 25%. (Naved *et al.*, 1990). There have been suggestions that the reduction of over 65% in ODAP content could be considered safe, the non toxic levels have not yet been established (Tekle-Haimanot *et al.*, 1993)

Naved *et al.* (1990) suggest that Khesari if prepared and consumed in the above manner, will not produce lathyrism and therefore no need to stop or ban the production of Khesari, so favorite a crop of the country, particularly of the poor.

1.10.2 Improvement of Khesari through breeding

The method involved selection and breeding for low toxin lines. Screening of germplasm has resulted in identifying several lines that have low toxin content (Jeswani *et al.*, 1970; Nerker, 1972; Samyajulu *et al.*, 1979; Ramnujam *et al.*, 1980). Lines are now available having 0.014% neurotoxin (BOAA), as compared to an average toxin of 0.8% (Roy and Bhat, 1976; Campbell and Deshpande, 1995).

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The pulse improvement programme at BARI has launched a concerted programme of genetic detoxification. The local germplasm was analysed for protein and BOAA/ODAP content to find out if there was any relationship between them. Similarly, studies were conducted to find out if ODAP/BOAA is associated with any morphological characters. If so, such associations can be used as markers for breeding and genetic studies.

Chemical analysis and planting tests indicated that same varieties or species grown in different locations with different climates has almost the same amount of ODAP. According to these results the environmental effects on the toxin of *Lathurus sativus* is negligible, the level of toxin being regulated by genes rather than by climate or soil. This implies that introduction of lower toxin varieties in different location with different climate and soil should not pose any problem of unstable toxicity. This widens the adaptive range of selective varieties with low toxin (Zhong, 1995).

Correlation studies between ODAP content and yield components indicated that there was no correlation between them except between ODAP and days to maturity. This is an indication of the possibility of developing strains low in neurotoxin by selecting early maturing varieties (Kaul *et al.*; 1982).

In Bangladesh Dutta *et al.* (1982) found some exotic genotype of *Lathyrus* were found to be low in neurotoxin but showed less adaptation to our climate condition. Ali *et al.* (1989) developed lower neurotoxin content mutants through gamma-irradiation but these were low-yielding.

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Shaikh *et al.* (1992) developed through hybridization programme recombinant with higher yield but low neurotoxin. The exotic and mutant germiplasms, G-47 and G-49 were crossed with the local lines L-1, L-6 and L-14. Selection were made in the F_1, F_2, F_3, F_4, F_5 & F_6 generations on the basis of ODAP and protein contents and seed yield. Finally. two lines have been identified as having lower ODAP with modest yield and protein content. In F_6 generation the line numbers 1 (G-49XL-1) and 2 (G49 X L-1) contained 0.125% and 0.06% of ODAP compared to 0.05% and 0.41% in G-49 and L-1 respectively. On the other hand, protein contents in lines 1 and 2 were 28& and 29.3% compared to 29.0% and 30.1% in G-49, end L-1 respectively. It is expected that either one or both of these two lines may be released as varieties in future.

1.11 Khesari production in Bangladesh

Khesari is cultivated in Bangladesh since time immemorial. Farmers generally use their own seed for the cultivation of this crop. It is obvious that evolutionary forces are operating on this crop (Sarwar *et al.*, 1995). Khesari accounts for one third of total pulses production in Bangladesh. Its excellent adaptation to rice-based cropping system, positive effect on soil health and its nutritious green fodder make it indispensable for sustainable agriculture in this country. No other species can replace this wonderful crop in the low lying areas.

Among pulses, Khesari ranks first in area and production (Table-2)

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Table 2: Area and production of different pulses in Bangladesh during 1990-91.

Crop	Area (ha)	Production (metric tons)	Yield kg/ha	% of Total	
				Area	Production
Khesari	238,992	182,046	762	35.02	36.78
Lentil	210,1164	159,119	757	30.79	32.15
Chick Pea	102,913	69,502	675	15.08	14.04
Black gram	68,587	57,710	754	10.05	10.45
Mungbean	60,125	31,287	520	8.81	6.32
Other Pulses	1,664	1,205	724	0.2	0.24

Source: Hamid (1993).

From latest information of Bangladesh Bureau of Statistics in 1992-93 the total production of Khesari was in 1st position by producing 172 thousand MT the production of Masur was in the second position being 163 thousand MT.

The NGO,s and sometimes Government media have been campaigning against the cultivation and consumption of Khesari since 1980. Despite this campaign, area under khesari has increased by 3-5% in 1991-92 compared to that in 1983-84 (Hamid 1993). The main reasons are that it is the hardiest among pulse crops, it has no major diseases or insect problems, it has stable yields, it requires no major input costs. It is easy to cultivate (relay cropping). It is difficult to replace it by any other crop under the situations in which it is presently cultivated. Realizing its importance in the cropping systems, Bangladesh Agricultural Research Institute (BARI) launced a breeding programme on this crop in 1979 to develop low toxin, high yielding cultivar, suitable for existing cropping system (Rahman *et al*,. 1995). In spite of official and un-official discouragement this crop is still grown in several parts of the world.

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In Pakistan *Lathyrus sativus* is grown over an area of 140 thousand hectares every year and the production is around 70 thousand metric tonnes per annum, (Agriculture Statistics of Pakistan, 1989-90).

In India *Lathyrus* is mainly grown in rice base cropping system as paira crop occupying an area of 1.5 million hectares with an annual production of 0.8 millions metric tons (Pandey and Kashyap, 1995).

In Ethiopia out of 13% of the total land area used for crop production, the amount of legume production contributed 7-13% of total crop production and occcupied 11-13% of cultivation area. Grass pea (*Lathyrus sativus*) is one of the important food legumes cultivated in high lands of Ethiopia (Seme, 1989).

1.12 Purpose of the study

The picture of malnutrition in Bangladesh is very shocking with a wide prevalence among all population groups, particularly the growing children. It adversely affects both mental and physical development of children and reduces their ability to resist diseases. They suffer from kwashiorkor and other protein related diseases. Severely affected mothers produce low birth weight babies who often do not survive the hostile infectious environment in which they are born and brought up. As adult labour forces they become less productive. Substantial school drop-outs, higher student repeater rate at primary level, early death, reduction of working hour of labour force resulting from malnutrition and nutrient deficiency diseases greatly reduce the return from the investments in health, education and other social sectors necessary to enable a person to develop his particular skill.

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Per capita income is the lowest as compared to that of developing and developed countries. This may be due to the lowest per capita supply of calorie and protein. Therefore malnutrition has negative influence on economic development of our country.

The problem of protein malnutrition among infants and young children is well recognized in Bangladesh. Here the intake of protein alarmingly declined during the last decades. The major portion of our population could not afford money to get adequate amount of animal protein due to its scarcity and high cost. To combat the prevailing protein problem, production of egg, milk and other quality protective food is inadequate, the other diets consumed here are generally deficient in proteins. This problem can be overcome by using more plentiful and cheaper plant proteins.

Among the plant proteins sources grown in Bangladesh pulses contain considerable amounts of protein. Legumes are nutritionally important among vegetable food because of their relatively high protein content. They are also less expensive. *Lathyrus* (Khesari) is in advantageous position in the context of its protein content and availability. So *Lathyrus* can be introduced as an optimistic protein source to minimize the existing protein gap in our country.

The objectives of present work are

1) to prepare a protein concentrate (*Lathyrus* protein concentrate, LPC) from *Lathyrus sativus* (Khesari)
2) to measure the nutritive value of the LPC, and
3) to study the possibility of introducing it as a supplementary food for mass consumption.

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Chapter 2

MATERIALS AND METHODS

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2. MATERIALS AND METHODS

2.1 *Lathyrus* protein concentrate (LPC)

2.1.1 *Lathyrus sativus* seed (Khesari)

The seed was collected from local market and powdered by a grinding machine. The powder was then stored at 4-10°C before being used.

2.1.2 *Lathyrus* protein extraction

The method of Gheyasuddin *et at.* (1970) was followed to isolate the protein from *Lathyrus sativus*. The lathyrus powder was suspended in water (1:10, W/V) and adjusted to pH 11.0 by the addition of IN NaOH and the mixture was stirred for 1 h at 20°C, then contrifuged at 12,000 r.p.m. for 10 min at 10°C. The supernatant was filtered to remove some floating materials and adjusted to pH 4.5 with IN HCl. The resultant precipitate was separated by centrifugation and washed with distilled water. A further washing reduced the toxin content to negligible trace amounts. The *Lathyrus* protein concentrate was then dried in an oven overnight at 40-45°C. The preparation of LPC is shown in flow chart below (Fig. 1). LPC was virtually free of the toxin ODAP.

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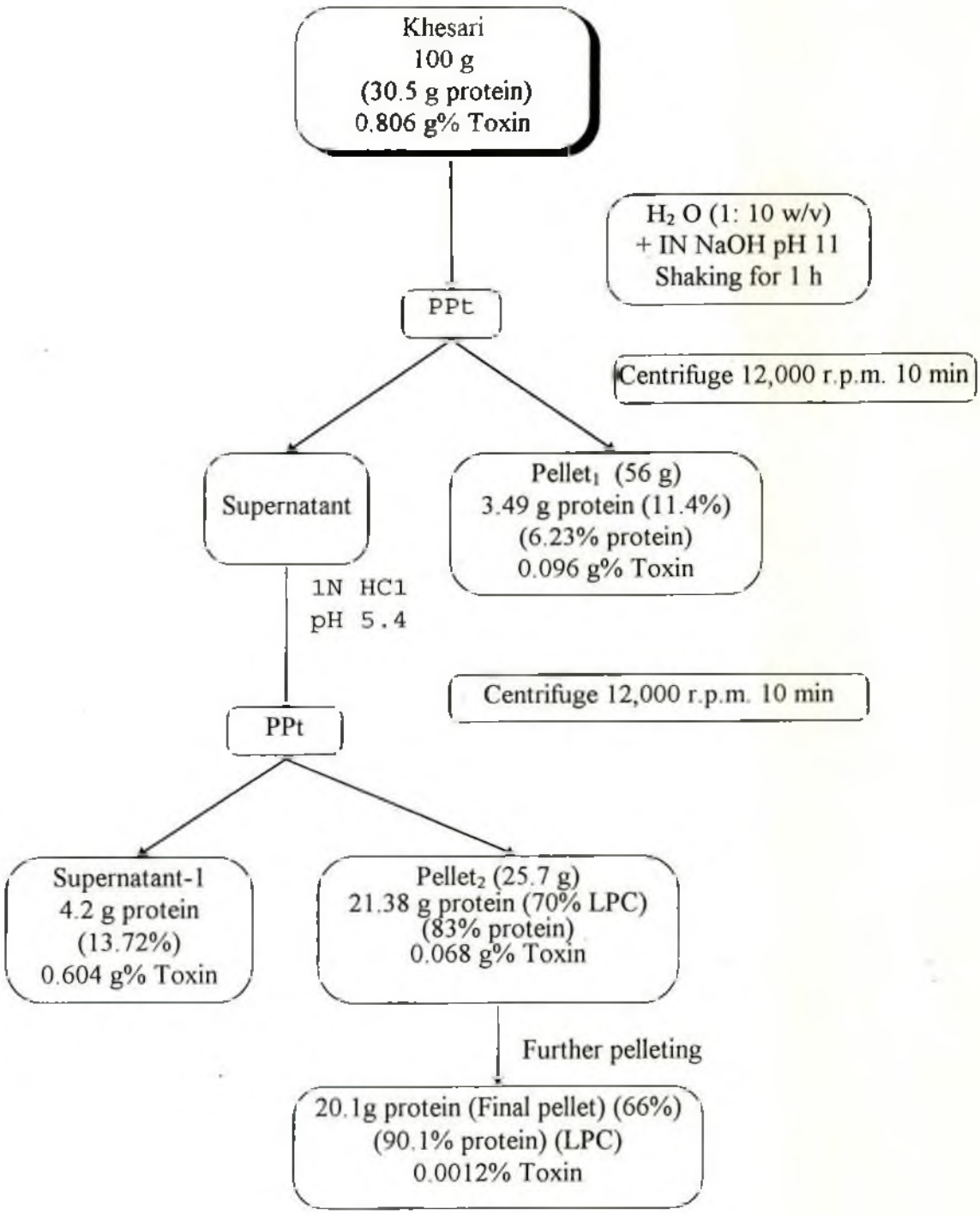

Fig. 1. Flow sheet of the method for the preparation of *Lathyrus* protein concentrate (LPC).

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2.2 Preparation of diets

2.2.1 Materials

The extracted *Lathyrus* protein concentrate (LPC) was dried and then powdered. Skim milk powder (Milk Vita) was a sample from the Eastern Milk producers co-operative society, Dhaka. Rice was purchased from local market and powdered by grinding machine. Vegetable oil of Jony brand was also purchased from local market. Starch, vitamins, minerals and all other ingredients were purchased from the different chemical stores. Each diet was mixed by mixing apparatus.

2.2.2 Diets

The experiment was designed to determine the nutritive value of *Lathyrus* protein concentrate (LPC) and that of rice + LPC. The experiment was divided into two phases, experiment 1 and expriment 2. The purpose of this division was to introduce different percentage of proteins in the experimental diets. According to plan of study two diets with *Lathyrus* protein concentrate was prepared: one with LPC as the sole source of protein named LPC diet and other was prepared in combination with rice powder, named as rice+LPC diet. The other diets were skim milk protein diet (used as reference diet), rice protein diet and the non-protein diet. The non-protein diet was prepared to determine the BV and NPU of experimental diets. The compositions of each diet for both experiment 1 and experiment 2 have been shown in Tables 5 to 13 respectively. The composition of supplemented vitamins mixures and salt mixtures also have been shown in Tables 3 and 4 respectively.

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Table 3: Composition of the vitamin mixtures.

Constituents	Amount (gm)
Thiamin hydrochloride	0.50
Riboflavin	0.50
Nicotinamide	2.50
Ca-pantothenate	2.00
Pyridoxine HCL	0.25
Folic acid	0.02
Vit.K (Menadione)	0.05
Biotin	0.01
Cyanocobalamine	0.002
Inositol	10.00
Ascorbic acid	5.00
Starch	227.17

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Table 4: Composition of the salt mixtures.

Constituents	Amount per kg diet
Calcium Gluconate	18.88
K_2HPO_4	6.01
Mg So4, $7H_2O$	5.07
KCl	4.38
NaCl	12.60
CuSO4, 5 H_2O	20.0
FeSO4, 7 H_2O	100.0
MnSO4, 4 H_2O	203.0
NaF	0.09
KI	0.30
$ZnCO_3$	30.0
Sodium molybadate, $2H_2O$	0.10
SeO_2	0.10
Cr $(SO_2)_3$, $15H_2O$	0.50

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Table 5: Composition of diet I.

(non-protein)

(Experiment-1)

Ingredients	**Amount (per kg)**
Starch, g	890
Vitamin mixture*, g	10
Salt mixture*, g	50
Soybean oil, g	50
Vit.E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.5

*Vit. and salt mixtures were taken from the composition as mentioned in Tables-7 and 8.

**Vit. E (α-tocopherol acetate) mixed with 10 g soybean oil, Vit. A (retinyl palmitate) and Vit. D mixed with 2 g soybean oil.

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Table 6 : Composition of diet II.

(18% skim milk protein)

(Experiment-1)

Ingredients	**Amount (per kg)**
Starch, g	288.0
Vitamin mixture*, g	10.0
Salt mixture*, g	50.0
Skim milk, g	601.2
Soybean oil, g	50.0
Vit. E**, mg	10.0
Vit. A**, I.U	600.0
Vit. D**, µg	1.5

*Vitamin and salt mixtures were taken as mentioned in diet I.

**Same as mentioned in diet I.

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Table-7: Composition of diet III.

(18% LPC Protein)

(Experiment-1)

Ingredients	Amount (per kg)
Starch, g	690.0
Vitamin mixture*, g	10.0
Salt mixture*, g	50.0
Lathyrus protein concentrate (LPC), g	200.0
Soybean oil, g	50.0
Vit. E**, mg	10.0
Vit. A**, I.U.	600.0
Vit. D**, µg	1.5

*Vitamin and salt mixtures were taken as in diet I.

**Same as in diet I.

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Table 8: Composition of diet IV.

(6.23% rice protein)

(Experiment-1)

Ingredients	Amount (per kg)
Rice powder, g	890.0
Vitamin mixture*, g	10.0
Salt mixture*, g	50.0
Soybean oil, g	50.0
Vit. E**, mg	10.0
Vit. A**, I.U.	600.0
Vit. D**, µg	1.5

*Vitamin and salt mixtures were taken as in diet I.

**Same as in diet I.

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Table 9: Composition of diet V.
(5.95% rice protein + 3% LPC)
(Experiment-1)

Ingredients	Amount (per kg)
Rice Powder, g	856.7
Lathyrus protein concentrate (LPC), g	33.3
Vitamin mixture*, g	10.0
Salt mixture*, g	50.0
Soybean oil, g	50.0
Vit. E**, mg	10.0
Vit. A**, I.U.	600.0
Vit. D**, µg	1.5

*Vit. and Salt mixtures were taken as mentioned in diet 1.

**Same as in diet 1.

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Table 10: Summary of the compositions of diets with or without *Lathyrus* protein concentrate (Per kg).

(Experiment-1)

Ingredients	Diet I Non-Protein	Diet II 18% milk protein	Diet III 18% LPC	Diet IV 6.23% Rice protein	Diet V 8.95% (rice protein 5.95% + LPC 3.0%)
Starch, g	890	290	690	-	-
Rice Powder, g	-	-	-	890	856
Skim-milk, g	-	600	-	-	-
LPC, g	-	-	200	-	33.3
Soybean Oil, g	50	Same as diet I	Same as diet I	Same as diet I	Same as diet I
Vit. E*, mg	10	10	10	10	10
Vit. A*, I.U.	600	600	600	600	600
Vit. D**, µg	1.5	1.5	1.5	1.5	1.5
Vitamin mixtures	10	10	10	10	10
Salt mixtures	50	50	50	50	50

* Vit. E (α-tochopherol acetate) mixed with 10g soybean oil, vit. A (retinyl palmilate) and Vit. D mixed with 2g soybean oil.

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Table 11: Composition of diet I.

(Non-protein)

(Experiment-2)

Ingredients	Amount (per kg)
Starch, g	890
Vitamin mixture*, g	10
Salt mixture*, g	50
Soybean oil*, g	50
Vit. E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.5

*Salt and vitamin mixtures were taken from the composition as in tables 7 and 8.

**Vit. E (α-tochopherol acetate) mixed with 10 g soybean oil, Vit. A (retinyl palmitate) and Vit. D mixed with 2 g oil.

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Table 12: Composition of diet II.

(6.5% skim milk protein)

(Experiment-2)

Ingredients	Amount (per kg)
Starch, g	672.9
Vitamin mixture*, g	10.0
Salt mixture*, g	50.0
Skim-milk, g	217.1
Soybean oil, g	50
Vit. E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.5

*Vitamin and salt mixtures were taken as in diet I

**Same as in diet I

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Table 13: Composition of diet III.

(6.5% LPC)

(Experiment-2)

Ingredients	Amount (per kg)
Starch, g	817.79
Lathyrus protein concentrali (LPC), g	72.20
Vitamin mixture*, g	10
Salt mixture*, g	50
Soybean oil, g	50
Vit. E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.5

, Same as in diet I

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Table 14: Composition of diet IV.

(6.5% rice protein)

(Experiment-2)

Ingredients	Amount (per kg)
Rice powder, g	890
Vitamin mixture*, g	10
Salt mixture*, g	50
Soybean oil, g	50
Vit. E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.5

*,** Same as in diet I

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Table 15: Composition of diet V.

(5.2% rice protein and 1.3% LPC)

(Experiment-2)

Ingredients	Amount (per kg)
Starch, g	132.70
Vitamin mixture*, g	10.00
Salt mixture*, g	50.00
Rice powder, g	742.85
Lathyrus protein concentrate (LPC), g	14.45
Soybean oil, g	50
Vit. E**, mg	10
Vit. A**, I.U.	600
Vit. D**, µg	1.50

*,** Same as in diet I

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Table 16: Summary of composition of diets of different groups of rats. (Experiment-2)

Ingredeints	Diet I Non-protein	Diet II 6.5% skim-milk protein	Diet III 6.5% LPC	Diet IV 6.5% rice protein	Diet V 5.2% rice protein + 1.3% LPC
Starch, g	890	672.9	817.79	-	132.8
Skim-milk, g	-	217.9	-	-	-
LPC, g	-		86.71	-	14.45
Rice, g	-		-	890	742.85
Soybean Oil	50	Same as diet I	Same as diet I	Same as diet I	Same as diet I
Vit. E*, mg	10	10	10	10	10
Vit. A*, I.U.	600	600	600	600	600
Vit. D*, µg	1.5	1.5	1.5	1.5	1.5
Vitamin mixtures, g	10	10	10	10	10
Salt-mixtures, g	50	50	50	50	50

* Vit. E (α-tochopherol acetate) mixed with 10g soybean oil, vit. A (retinyl palmilate) and Vit. D mixed with 2g soybean oil.

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2.3 Animals and grouping

Adult male Long-Evans strain rats of 4-8 weeks of age weighing between 66 - 158 g were purchased from the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B). Sixty rats were used throughout the experiment (experiment 1 and experiment 2). They were given a standard basal diet for few days for their adaptation in a new environment.

After adaptation, the rats were divided into five groups each having 5-6 rats (except in non-protein group in experiment in which had 10 rats).

The rats received the following diets according to their grouping for sixweeks (experiment 1) and 4 weeks (experiment 2).

1. Non-protain diet
2. Skim-milk protein diet
3. LPC diet
4. Rice protein diet
5. Rice protein + LPC diet

2.4 Food intake

Daily food intake of all animals were measured to determine the food efficiency of the diets in each group. The records of the food given and the left over were maintained to assess the actual consumption of the diet.

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2.5 Growth pattern

The body weight of the animals were taken twice a week to determine the growth pattern of the rats of all groups.

2.6 Collection of feces and urine

Each rat of each group was housed in a metabolic cage for 24 hours. Their feces and urine were collected for determination of excreted protein.

2.7 Collection of blood serum

Blood was collected from teh rats from the tail and centrifuged to separate the serum. Serum was used to determine lipid profile and total protein.

2.8 Chemical analysis of *Lathyrus sativus*

2.8.1 Determination protein content of LPC

The content of nitrogen in LPC was measured by Kjeldahl method according to Campelli and Hanna (1932), which depends on the fact that organic nitrogen present in the sample when digested with concentrated H_2SO_4 in the presence of a catalyst is converted into ammonium sulfate. By steam distillation in the presence of strong alkali, ammonia is liberated which can be estimated by distilling into a known volume of standard acid which is then back titrated.

Reagents

(a) Concentrated sulphuric acid

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(b) Catalyst: A mixture of K_2SO_4 and $CuSO_4$ (98:2, w/w).

(c) Sodium hydroxide solution (40%): 40 g NaoH was dissolved in distilled water to make the volume 100 ml.

(d) 0.1 N sulphuric acid: 2.71 ml conc. H_2SO_4/100 ml distilled water.

(e) 0.1 N sodium hydroxide: 4 g NaOH/100 ml distilled water.

(f) Methyl red indicator: Prepared by dissolving methyl red indicator (0.1 g) in 60 ml of alcohol and water was added to make the volume 100 ml.

Procedure

Samples (2.0 g) were taken into a dry kjeldahl flask. Pure concentrated sulphuric acid (20 ml) and catalyst (about 5.0 g) were added to the flask. Glass beads were also added to prevent bumping. The contents heated for 4 to 5 hours, until the solution became clear. The heating was continued for at least one more hour. The contents were allowed to cool and transfered with distilled water into a distillation flask and the contents made alkaline by adding exess of 40% NaoH in the presence of the litmus paper. A small quantity of pumic powder was added to the distillation flask to prevent bumping before distillation. The ammonia liberated during distillation is distilled into a receiver containing 50 ml of sulphuric acid (0.1 N). The exess of acid in the receiver is back titrated against sodium hydroxide (0.1 N) using 2-3 drops of methyl red indidcator.

A reagent blank was similarly digeted and distilled without using any sample. This titre value is substracted from the value obtained for the sample to get the true value.

Calculation

Based on stochiometric relationship involved in the titration, the amount of protein in the sample can be calculated according to the formula,

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$$\text{Protein content (g/100g)} = \frac{(C-B) \times 14 \times D \times 6.25 \times 100}{A \times 1000}$$

where, A = Weight of sample

B and C = Volume of alkali (0.1 N) required for back titration of blank and sample solution respectively and to nutralize 50 ml of 0.1 N sulphuric acid.

d = Strength of the alkali.

2.8.2 Determination of protein purity of LPC

The purity of protein in the *Lathyrus* protein concentrate was measured by kjeldahl method. In this method the total protein content of the extracted protein sample was measured. By subtracting this value from extracted protein, the amount of non-protein substances was determined. And the protein purity was determined by following equation.

$$\text{Purity \%} = \frac{\text{Extracted protein (mg)- Non- protein substances(mg)}}{\text{Extracted protein (mg)}} \times 100$$

2.8.3 Determination of toxin content of LPC

a) Quantitation by colorimetry

The ODAP content of Khesari seeds, the different precipitates and the final LPC was determined by the colorimetric OPT method of Rao (1978) DAP (alpha, beta-diaminopropionic acid, also called BAA, beta-amino-L-alanine) by an alkali (NaOH or KOH) and the DAP is then reacted upon with O-phthalaldehyde (OPT) to prodduce an intense yellow colour which obeys Beer's law and is therefore measurable spectrophotometrically. The reaction was carried out in 0.375M final

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concentration of potassium borate buffer, pH 9.9, for 30 min, followed by determination of absorbance at 420 nm.

The pH of he reaction mixture was strictly maintained at around 10.0 which was very critical for the reaction to take place (Fig. 2)

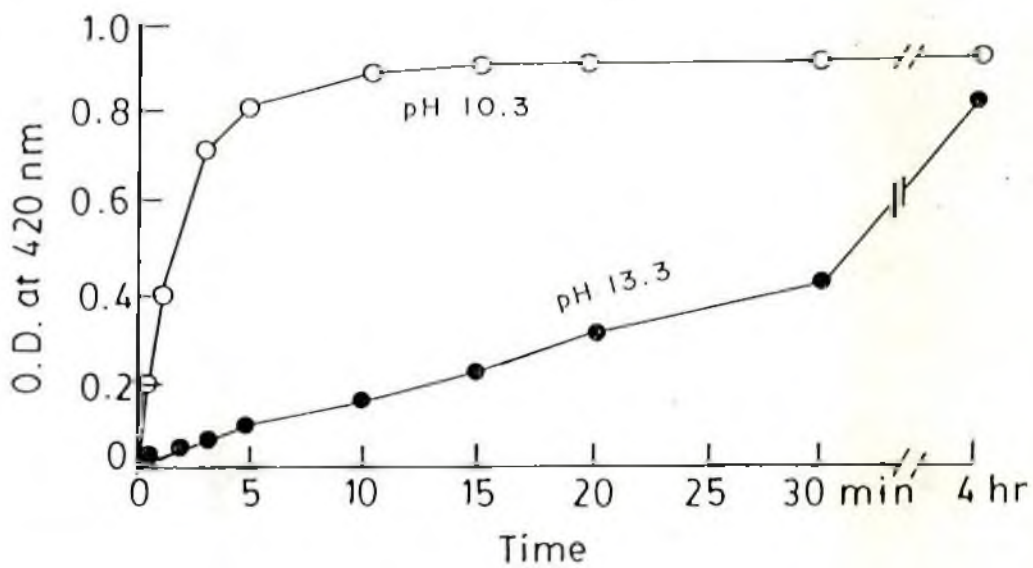

Fig. 2 Effect of pH on the speed and extent of DAP-OPT reaction, 0--0 pH, 10.3 ; •------•, pH 13.3. The reaction reaches near completion within 10 min after the reaction is started at pH 10.3, whereas at pH 13.3 it takes more than 4 hrs to reach completion.

The colorimetric OPT method was 3-4 times more sensitive than the paper chormatographic method, although the metasured values of the same samples by

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the two methods were fairly comparable. 3). The OPT method was therefore used subsequently in all further experiments.

b) Reagents and chemicals of the colorimetric method

i) ODAP standard (0.2 µg/ul): ODAP synthesized in our laboratory (95% pure) was dissolved in distilled water at the above concentration and stored at 4°C.

ii) Potassium borate buffer, 0.5M, pH 10.3.

iii) KOH solution, 3N in water.

iv) OPT reagent: OPT (200 mg) was dissolved in 1 ml 95% ethanol and to it was added 0.2 ml mercaptoethanol. The mixture was added to 99 ml of the boric acid buffer. The reagent was stable for 3 days, but if mercaptoethanol was added just before use, the reagent could be used for weeks.

c) Procedure of the colorimetric method

Finely powdered seed material (50 mg) was shaken for 6 hr with 5 ml 70% ethanol. The mixture was centrifuged at 3,000 x g for 5 min and the clear supernatant was used for quantitative determination. A set of screw-capped tubes were taken and filled with standard ODAP (20-100 µl = 4-20 µg ODAP). 0.2 ml of 3N KOH was added to each tube and the mixtures were kept in a boiling water-bath for 30 min. After cooling the tubes to room temperature, the volume in each tube was brought up to 1 ml by adding appropriate amount of distilled water. Then 2 ml of the OPT reagent was added. The absorbance of the yellow colour in each tube was measured after 30 min at 420 nm against a blank that contained everything but no ODAP.

For the samples, two sets of screw-capped tubes were taken. In one set (duplicate), 100-200 µl of the unknown extract were treated as above and in the

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other set (also in duplicate) same volume of the extract was treated as above but no KOH was included (i.e. in this set there was no hydrolysis of ODAP). The absorbance of samples without hydrolysis was very small and the difference between absorbance readings with and without hydrolysis gave an estimate of ODAP calculated from the standard curve.

2.8.4 Determination of protein composition of LPC by SDS polyacrylamide gel electrophoresis (PAGE)

a. Electrophoresis

Any charged ion or group will migrate when placed in an electric field. Since proteins carry a net change at any pH other than their isoelectric point they too will migrate and their rate of migration will depend upon the charge density (the ratio of charge to mass), the higher the ratio of charge to mass the faster the molecule will migrate. Thus protein in a mixture when analyzed will separate according to their size and charge. The mixture of proteins to be separated is placed as a narrow zone or band at a suitable distance from the electrodes. During electrophorese is proteins of different mobilities travel as discrete zone which gradually separate from each other as electrophoresis proceeds.

Details methodology vary with the type of gel and butter system selected. The chemicals and solution we used are described here.

b. Preparation of sample buffer for gel electrophoresis (sds-sample buffer)

Constituents	/100 ml
SDS	2.3 gm
ß-mercaptoethanol	5 ml
Glycerol	10 ml
Tris (pH 6.8)	0.75 gm
Bromophenol blue	0.1 gm

The final volume was made upto 100 ml and was stored in small aliquots in a freezer.

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c. Preparation of stock gel solution

i. Preparation of 30% acrylamide solution

	gm/200 ml
Acrylamide	60
Bis-acrylamide	1.6

The final volume was made upto 200ml and stored at 4.C in a brown bottle.

ii. Preparation of 10% SDS solution

10 gm of SDS dissolved in deionized water to produce 100 ml solution.

iii. Preparation of resolving gel buffer

	/200 ml
Tris (pH 8.8)	36 gm
10% SDS	8 ml

The final volume was made upto 200 ml and stored at 4°C.

iv. Preparation of stacking gel buffer

	/200 ml
Tris (pH 6.8)	12 g
10% SDS	8 ml

v. Preparation of 10% ammonium persulfate solution

2 gm of ammonium persulfate was dissolved in deionized water to produce 20 ml solution. This solution was stored at -20°C in eppendrof tubes.

d. Preparation of Marker

i. Contents of SDS-7 protein were reconstituted with 1 ml of sample buffer. SDS-7 available from Sigma Chemical Company, USA consisted of Albumin, Bovine (66,000 MW); Albumin, Egg (45,000 MW); Glyceraldehyde-3-

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phosphate dehydrogenase (36,000 MW Subunit); Carbonic anhydrase, Bovine erythrocyte (29,000 MW); Trypsinogen, Bovine pancrease (24,000MW); Trypsin inhibitor, Soybean (20,100 MW); α-Lactalbumin, Bovine milk (14,200 MW).

ii. Another molecular weight markers, available from Sigma Chemical Company, USA, consisted of α-lactalbumin (14,200 MW); Carbonic anhydrase (29,000MW); Albumin, Chicken egg (45,000 MW); Urease, Jack been (272000 MW, trimer, 545000 MW, hexamer).

e. Preparation of electrophoresis buffer

	/2 L
Tri	6 g
Glycine	28.6 g
SDS (1%)	2 g

The final volume was made upto 2 litres with deionized water.

f. Composition of resolving or separating Gel

Percentage of gel	7.5%	8%	18%	13%
30% acrylamicd solution	11 ml	11.2 ml	25.2 ml	21.4 ml
Resolving gel buffer	10 ml	10 ml	10 ml	12 ml
Deionized water	21 ml	20.8 ml	6.8 ml	16 ml
10% APS	150 µl	100 µl	150 µl	150 µl
TEMED	10 µl	10 µl	10 µl	10 µl

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g. Composition of stacking Gel

Stacking gel buffer	2.5 ml
30% Acrylamide solution	1.4 ml
Deionized water	6.5 µl
10% APS	100 µL
TEMED	10 µL

h. Peparation of stain for PAGE

(Polyacrylamide Gel Electrophoresis)

	/L
Methanol	500 ml
Coomassie blue	2.5 gm
Acetic acid	100 ml

The final value was made upto 1 litre by addiging deionized water.

i. Preparation of stain for PAGE (Polyacryl amide gel electrophoresis)

	/L
Acetic acid (10%)	100 ml
Methanol (50%)	500 ml
Commassie blue (0.25%)	2.5 gm

2.5 gm of Commasie blue were taken in a beaker, the above mentioned acetie acid and methanol mixed. Commassie blue was dissolved well in this mixture and the volume was made upto 1000 ml by adding distilled water.

j. Preparation of destain

	/2 L
Acetic acid (10%)	200 ml
Methanol (50%)	1000 ml

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The above mentioned constituents were taken in a 2000 ml beaker and made the final volume made upto 2000 ml with distilled water.

k. An electrophoretic run (Hames, 1986)

The glass plates were cleaned, washed with acetone, dried and assembled with spacers. The plate assembly was clamped together with strong metal clips which are possible to press on the same which just over the spacer positions.

Appropriate volume of separating gel mixture was prepared. About 3-4 ml was taken from this mixture and poured quickly with 40 µl APS and 10 µL TEMED to seal the bottom. The rest of the resolving gel buffer mixture was degased, 175 mL APS and 40 mL TEMED was added, gently mixed and poured in to the glass plate leaving sufficient space at the top for a stacking gel. The gel was overlayed with butanol and was kept undisturbed for 1hr. When the gel had polymerized, the butanol was drained off, washed with distilled water 4-5 times and dried. The stackign gel buffer was poured, the comb was inserted and allowed to set for 1hr.

Before loading, the samples were mixed with SDS-sample buffer, boiled for 3 minutes and centrifuged for 30 seconds (10,000 rpm) and supernatant was collected.

The comb was gently remove dand the slots were rinsed distilled water. 20-40 µL) samples were loaded per slots. BSA solution (10 µg/20 µL) and Dalton marker protein were used as standard.

The electrophoretic chamber was filled with running buffer and the gel plate was installed in the apparatus. It is essential to remove any air bubbles from the bottom of the gel. Running buffer was added to the top of the chamber. Electrical supply was connected and set to 35 m Amp constant current and 200 V. When

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the dye front had reached at the bottom (required about 3-4 hrs), the gel was removed, immersed in to the staining solution for 1-2 hrs and destained over night.

2.8.5 Determination of amino acid composition of LPC

This analysis was done in the laboratory of professor Tadashi Ogawa, Department of Nutrition, Tokushima university, Japan.

Protein and peptide were hydrolyzed in 5.7 N HCl containing 0.02% 2-mercaptoethanol at 110°C for 24h in evacuated sealed tubes. The amino acids were analyzed on a Shimadzu LC6A system amino acid analyzer after derivatization of amino acids with PITC, as described by Heirrikson and Meredith (1984).

2.8.6 Determination of digestibility of LPC

The digestibility of LPC was determined by the method of Walter (1964). In this method protein was digested by pepsin followed by pancreatin. Digestion index is one of the major phenomena to evaluate protein quality.

a. Preparation of pepsin solution

Pepsin solution was prepaed in 0.1 N HCl. Pepsin (20 mg) was weighed accurately and dissolved in 0.1 N HCl and volumed up to 150 ml. This pepsin solution was preserved at 0-4°C.

b. Preparation of 0.2 N NaOH

NaoH (800 mg) weighed separately and was dissolved in 100 ml of distilled water.

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c. *Preparation of Na-phosphate buffer solution containing lmM $CaCl_2$ and 0.01% NaN_3*

The buffer was prepared by dissolving 6.15 g Na_2HPO_4. $12H_2O$ and 0.42 g HaH_2PO_4. $2H_2O$ in 80 ml of distilled water. 11 mg $CaCl_2$ and 10 mg NaN_3 were weighed separately and dissolved in the above buffer. The final volume was made to 100 ml by distilled water. After adjusting the pH the buffer solution was stored in a refrigerator (0-4°C).

d. *Preparation of pancreatin solution*

To prepare a pancreatin solution, 4 mg of pancreatin was dissolved in 7.5 ml of Na-phosphate buffer containing 1mM $CaCl_2$ and 0.01% NaN_3.

e. *Preparation of 20% TCA solution*

Trichloro acetic acid (40 g) was weighted accurately and taken in a 500 ml beaker and the proper volume (200 ml) was achieved by adding distilled water.

f. *Procedure for in vitro digestion*

A portion of the LPC (about 200 mg) was suspended into 15 ml of pepsin solution in a 100 ml beaker. The suspension was incubated for 3 hours at 37°C in an incubator. After 3 hours the beaker was taken out from the incubator and 7.5 ml of 0.2 N NaOH was added to shift the pH to about 7.5 to 8.0. After that 7.5 ml pancreatin solution was added. The beaker was again incubated for 24 hours in an incubator at 37°C for digestion to occur. The undigested protein was precipitated by adding 30 ml of 20% TCA solution. This undigested protein was separated by whatman 41 filter paper. The amount of undigested protein was measured by kjeldahl method. Thus the digestibility of the LPC was determined from the following

$$\text{Digestibility\%} = \frac{\text{Total protein - undigested protein}}{\text{Total protein}} \times 100$$

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2.9 Determination of nutritional value of LPC

2.9.1 Determination of protein efficiency ratio (PER)

Sixty rats from Long-Evans strain aged about 4-6 weeks, body weight 68-125 g, were used for the study. Rats were grouped as mentioned earlier (section 2.4) and each rat was housed in a separate cage. Diet and water were given *ad libitum*. Body weights of the rats and residual diets were measured twice in a week.

The feeding continued for 28 days. PER values were calculated according to the method of Osborn *et al.*, (1919).

$$PER = \frac{\text{Gain in body wt. in gm}}{\text{Protein intake in gm}} = \text{gain of body weight per g of protein consumed.}$$

2.9.2 Determination of biological value (BV)

Biological values were calculated according to the method of Allison (1964). The test are mostly based on estimation of nitrogen balance. The duration of feeding was ten days.

$$BV = \frac{\text{Retained nitrogen}}{\text{Absorbed nitrogen}} \times 100$$

$$= \frac{I_N - (F_N - U_N)}{I_N - F_N}$$

The measured fecal & urinary nitrogen was corrected by subtracting the quantities lost in a protein free diet.

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$$BV = \frac{I_N - (F_N - F_O) - (U_N - U_O)}{I_N - (F_N - F_O)} X\ 100$$

I_N = Nitrogen intake

F_N = Fecal nitrogen output on the experimental protein diet.

U_N = Urinary nitrogen output on the experimental protein diet.

F_O = Fecal nitrogen output on a protein free diet.

U_O = Urinany nitrogen output on a protein free diet.

2.9.3 Determination of net protein utilization (NPU) value

The NPU was calculated according to the method of Allison (1964). The feeding time was ten (10) days.

$$NPU = \frac{\text{Retained nitrogen}}{\text{Intake of nitrogen}} X\ 100$$

$$= \frac{I_N - (F_N - U_N)}{I_N}$$

The measured fecal & urinary nitrogen was corrected by subtracting the quantities lost in a protein free diet.

$$NPU = \frac{I_N - (F_N - F_O) - (U_N - U_O)}{I_N} X\ 100$$

I_N = Nitrogen intake

F_N = Fecal nitrogen output on the experimental protein diet.

U_N = Urinary nitrogen output on the experimental protein diet.

F_O = Fecal nitrogen output on a protein free diet.

U_O = Urinany nitrogen output on a protein free diet.

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2.9.4 Determination of serum cholesterol

Seurum total cholestrol was dertermined enzymatically according to the method of Allian *et al.* (1960).

Reagents

(a) Enzyme-buffer solution

Phosphate buffer	0.1 mol/1
Phenol	15 mmol/1
Sodium cholate surfactant	3.74 mmol/1
4- aminoantipyrine	0.5 mmol/1
Peroxidase	≥ 1000 U/l
Cholesterol oxidase	≥ 200 U/l
Cholesterol esterase	≥ 125 U/l

(b) Standard cholesterol solution 200 mg/dl

Procedure

Duplicate sample (10 ml) of serum and standard of cholesterol containing 0 to 20 mg cholesterol were taken in Eppendrof tubes. Enzyme-buffer solution (1.0ml) was added to each tube and the contents were mixed vigorously. The tubes were incubated for 10 minutes in a water bath at 37°C. After incubation, the absorbance was read at 500 nm against corresponding blank. A standard curve of cholesteriol was prepared by plotting the absorbance values against the corresponding amount of cholesterol.

2.9.5 Determination of serum HDL cholesterol

Serum HDL cholesterol was separated and determined by the method of Burstein *et al.* (1970).

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Reagent

(a) *Enzyme-buffer solution:* Discussed previously.

(b) *Precipitating reagent:*

Phosphotungstic acid	40 g/l
$MgCl_2$ $6H_2O$ pH 6.2	100 g/l

(c) *HDL cholesterol calibrating solution:*

Free + esterified cholesterol	1.30 mmol/1

Precedure

Serum (0.5 ml) was pipetted into a Eppendrof tubes containing precipitating reagent (0.05 ml), mixed well and kept for 10 minutes at room temperature. The tubes were then centrifuged at 5,000 rpm for 15 minutes in a eppendrof centrifuge to precipitate the chylomicrons and lipoproteins of very low density (VLDL) and low density (LDL) contained in the sample. Duplicate sample of supernatant (0.05 ml) were then taken into eppendrof tubes. Enzyme- buffer solution (10 ml) was added to each tube, mixed and incubated for 5 minutes at 37°C. For standard, HDL-cholesterol calibrating solution (0.05 ml) was added with enzyme buffer solution (1.0 ml) and for blank distilled water (0.05 ml) was added to enzyme-buffer solution (1.0 ml). Both standard and blank were mixed well and stand for 5 minutes at 37°C. The absorbance was read at 520 nm against corresponding blank.

Calculation

Serum HDL-cholesterol content is calculated according to the formula given below.

$$\text{Serum HDL-cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times N$$

where n = 55 (accounts for serum dilution made for precipitation.)

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2.9.6 Determination of serum LDL cholesterol

Serum LDL-cholesterol was separated and determined by a method described by Steinberg (1981).

Reagents

(a) *Enzyme - buffer solution:* Discussed previously.

(b) *LDL precipitating reagent:*

Polycyclic anionic surfactant	0.4 g/l
Polycondensed polycyclic anioni surfactant	0.8 g/l
Polysubstituted dioxane	12.4 mmol/l
Immidazole buffer pH 6.10	25 mmal/l

(c) *LDL solubilizing reagent:*

Trisodium citrate	0.15 mol/l
Sodium chloride	0.11 mol/l

(d) *LDL cholesterol calibrating solution:*

Free + esterified cholesterol	1 mmol/l

Procedure

Serum (0.05 ml) was pipetted into a Eppendrof tube containing LDL-precipitating reagent (1.0 ml) and mixed well. The tubes were incubated for 30 minutes at 2-8°C and then centrifuged for 5 minutes at 4,000 rpm. The supernatant was drained off from the pellet by inverting the tubes and the separated LDL-cholesterol precipitate was solubilized by adding LDL solubilizing reagent (0.5 ml).

Duplicate samples of solubilized pellet (0.1 ml) were taken in to Eppendrof tube. Enzyme-buffer solution (1.0ml) was added to each tube, mixed well and incubated for 5 minutes at 37°C. For standard, LDL cholesterol calibarating solution (1.0 ml)

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was mixed with enzyme-buffer solution (1.0 ml) and for blank distilled water (0.1 ml) was mixed with enzyme-buffer solution (1.0 ml). Both standard and blank were treated in the same manner as the samples. The absorbance was measured at 520 nm against corresponding blank. The concentration of LDL - cholesterol was determined by the calculation given below.

Cadlculation

$$\text{Serum LDL - cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times n$$

Where n = 387 (accounts for serum dilution made for precipitation and for solubilization of pellet).

2.9.7 Determination of serum triglyceride

Serum triglyceride was determined enzymatically according to the method of Jacobs *et al.* (1960).

Reagents

(a) Enzyme - buffer solution:

PIPES buffer (PH 7.5)		40 mmol/1
Magnesium ions		5 mmol/1
4 - chlorophenol		5 mmol/1
Sodium azide		0.1 %
4 - aminoantiphrine		0.4 mmol/1
ATP		1.0 mmol/1
Lipases	≥	150 U/ml
Glycerol kinase	≥	0.4 U/ml
Glycerol-3-phosphate oxidase	≥	1.5 U/ml
Peroxidase	≥	0.5 U/ml

(b) Triglycerides standard: 200 mg/dl

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Procedure

Duplicate serum (0.1 ml), standard triglycerides solution (0.01 ml) were takent in Eppendrof tubes and enzyme - buffer solution (1.0 ml) was added to each tube. The contents were mixed well and incubated for 5 minutes in a water bath at 37°C. Enzyme - buffer solution treated in the same manner as above served as blank. Absorbance was measured at 500 nm against the regent blank. The concentration of triglyceride was calculated as follows:

$$\text{Serum triglyceride (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.9.8 Determination of serum total protein

Principle

The method described below is that of Lowry *et al.* (1951). The final colour is a result of:

a) Biuret reaction of protein with copper ion in alkali; and b)Reduction of the phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophan present in the treated protein.

Reagents

a) 4% Na_2CO_3

b) 0.5% $CuSo_4$. $5H_2O$ in 1 % sodium or potassium tartrate

c) Alkaline copper solution. Mix 50 ml of reagent A with 2 ml of reagent B. Prepare fresh every day.

d) Diluted Folin's reagent: Dilute Folin-Ciocalteau reagent (purchased from V.P. Chest Institute, Delhi) with an equal volume of O.1 N NaOH.

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Standard protein solution

The standard solution is prepared with bovine serum albumin to have a concentration of 100µg protein/ml.

NaoH: 0.1 N

Procedure

The test sample containing about 50 to 100 µg protein/ml is taken at three levels- 0.5, 1.0 and 1.5 ml. The necessary amount of 0.1 N NaoH is added to bring the volume to 1.5 ml. To this 1.5 ml of reagent `C' is added. Each tube is shaken and allowed to stand for 10 minutes, after which exactly 0.15 ml of diluted Folin's reagent added with continuous shaking. The tubes are allowed to stand for half an hour and then read at a wave length of 750 mµ. For more concentrated solutions, the readings may be kept in a workable range by reading it at a wavelength of 500 mµ.

The standard is prepared in the same way, the levels taken being 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml (25 µg to 150 µg protein).

The unknown sample is then read off the standard curve and the necessary calculations made.

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Chapter 3

RESULTS

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3. RESULTS

3.1 LPC and its nitrogen content

Protein was extracted from *Lathyrus* (Khesari) according to standard procedure and purified to a concentrate (*Lathyrus* protein concentrate, LPC) which was virtually free of toxin. As shown in Fig 1 the amount of LPC was about 20 g out of 30.5 g protein from 100 g Khesari (Table 17). So the protein recovery was about 66% (Table 18). The nitrogen content in LPC was 90% (Table 19).

Table 17: Protein content of Khesari seed (Kjeldahl).

Sample	Protein (%)
Khesari (Lathyrus sativus)	30.5

Table 18: Amount of extracted LPC and its percentage.

Sample name	Mean protein content	Extracted LPC g	% Extracted
Khesari	30.5	20.1	66

Table 19: Purity of protein in LPC Sample taken 500 mg

Sample name	Amount of protein mg	Purity %
LPC	450	90

1. Each value is the mean of 3 separate determinations

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3.2 ß-N oxalyl-L-α, ß-diamino propionic acid (ODAP) content in LPC

Table 20 represents the toxin content in different fractions during LPC preparation. Initially the *Lathyrus* (100 g) contained 806 mg of ODAP. The maximum quantity of ODAP was removed by supernatant and it was about 604 mg (0.604%) of starting value. Finally the LPC had only 1.2 mg (0.0012%) of toxin.

Table 20: Toxin (ODAP) content of different fractions during different steps of LPC preparation.

Fraction	Amount	*ODAP(mg/100g)	%
Khesari, g	100	806	0.806
$Pellet_1$, g	56	96	0.096
Supernatent1, ml	900	604	0.604
$Pellet_2$ (LPC), g	25.7	68	0.068
Final Pellet, g (Final LPC)	20.10	1.2	0.0012

* ODAP = ß N-Oxalyl L-α, ß-diamino propionic acid.

3.3 Protein composition of LPC

The proteins of the LPC were separated by SDS polyacrylamide gel electrophoresis. The LPC was separated into four major fractions of apparent molecular weights. 113,000, 100,000, 55000, and 24000 respectively (Fig. 3). All the protein fractions belong to the globulin family, as is found in other pulses. The electrophoretic pattern thus shows that there occured no internal polymerization of the LPC. during its preparation. Its digestibility (99%) also indicates absence of polymerized proteins which are rather difficult to digest.

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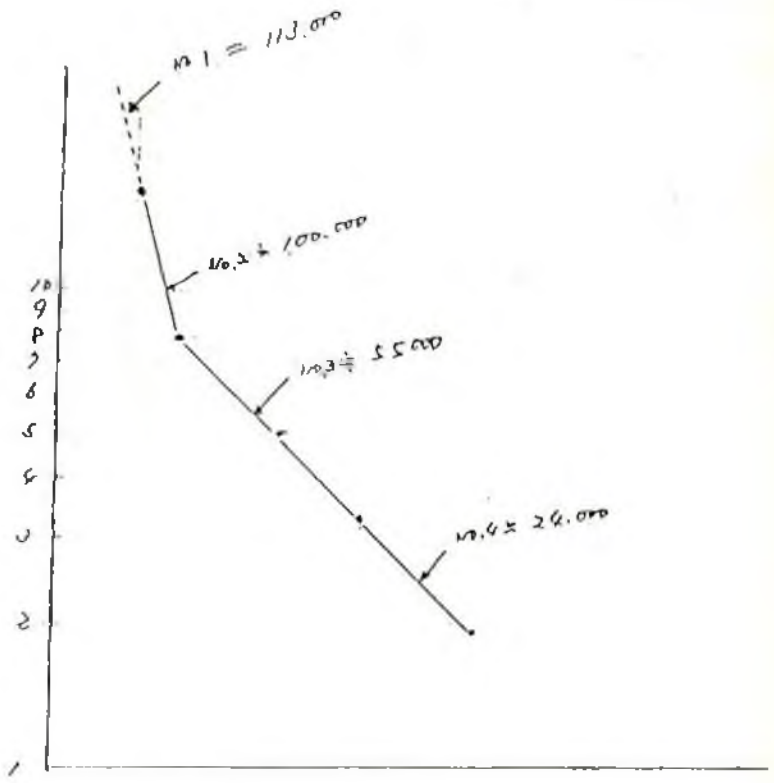

Fig. 3. Apparent molecular weights of the protein components of LPC separated by SDS-polyacrylamide gel electrophoresis.

3.4 Amino acid composition of LPC

Fig. 4 represents the separation of amino acids of LPC, by automated amino acid analyzer, and Table 21 represents the quantitative results of amino acids in LPC. As expected the sulphur containing amino acids in LPC were rather low, only 217 to 292 mg%. The moderately high quantity of lysine (4.06 g%) was present in LPC. All other amino acids were present in appreciable quantities.

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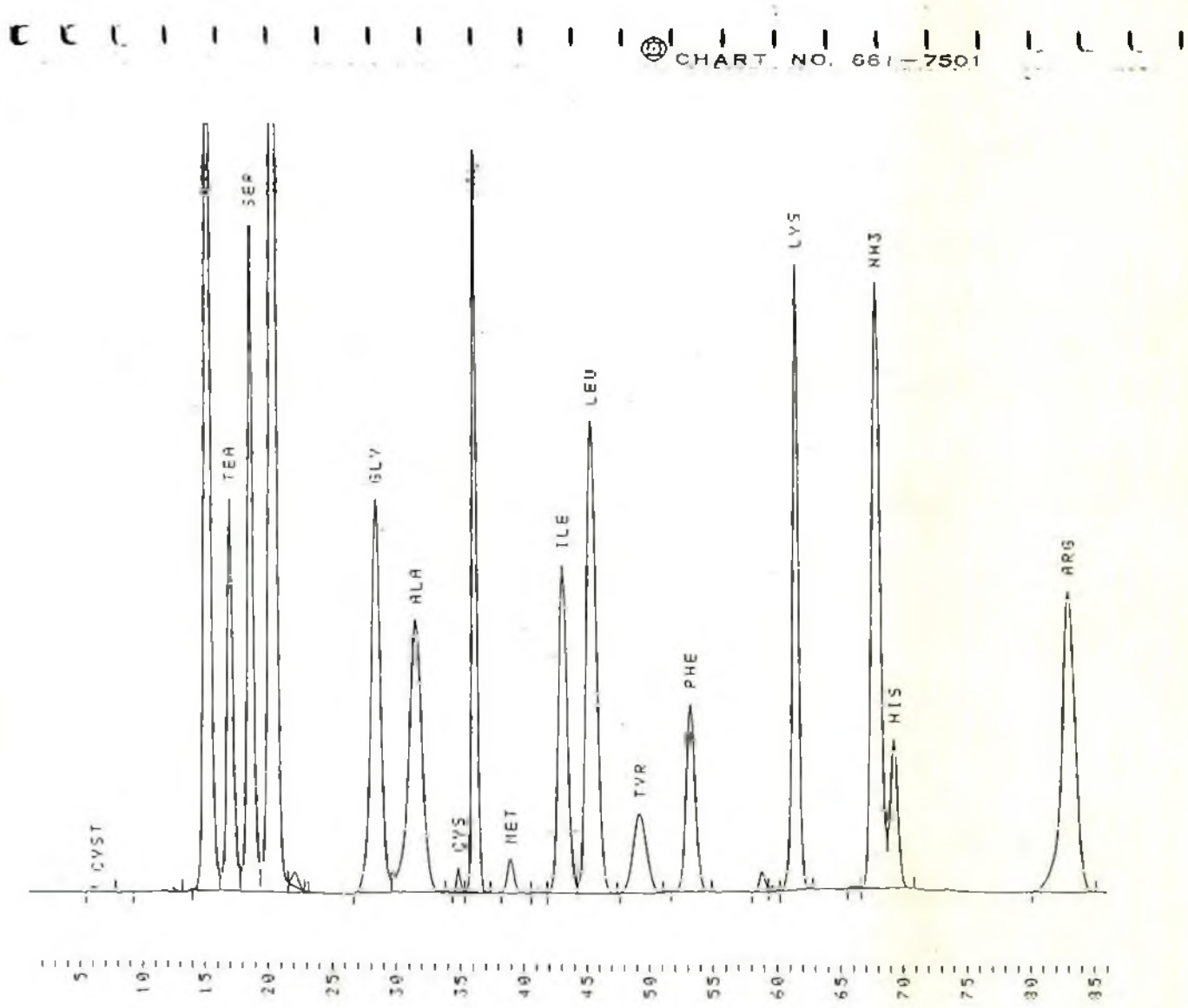

Fig. 4. Amino acids of LPC as separated by automatic amino acid analyzer.

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Table 21: Amino acid composition of *Lathyrus* protein concentrate (LPC).

Amino acid	nmol	R.W. (ng)	N	mg/100g
Asp	12.247	1409.5	171.5	7873.3
Thr	4.278	432.5	59.9	2460.9
Ser	6.780	590.4	95.0	3441.9
Glu	16.385	4115.6	229.5	11961.0
Pro	5.839	567.1	81.8	3246.2
Gly	7.389	421.6	103.5	2680.2
Ala	6.828	485.3	95.6	2938.6
1/2Cys	0.374	38.2	5.2	217.4
Val	6.701	664.3	93.9	3790.21
Met	0.406	53.3	5.7	292.7
Ile	5.307	600.5	74.3	3363.1
Leu	9.048	1023.9	126.7	5730.3
Tyr	2.190	357.4	30.7	1916.5
Phe	4.271	628.6	59.8	3408.0
Lys	5.750	737.0	161.1	4060.6
His	2.289	313.9	96.2	1716.1
Arg	7.362	1149.9	412.5	6194.5
NH3	15.026		210.4	
Trp	-			
Total		11588.7	1902.9	

Protein-nitrogen conversion factor : 6.09

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Table 22: Essential amino acid composition of *Lathyrus* protein concentrate (LPC).

Essential amino acids	n mol	R.W. (ng)	N	mg/100 g LPC
Arginine	7.362	1149.9	412.5	6194.5
Histidine	2.289	313.9	96.2	1716.1
Isoleucine	5.307	600.5	74.3	3363.1
Leucine	9.048	1023.9	126.7	5730.3
Lysine	5.750	737.0	161.1	4060.6
Methionine	0.406	53.3	5.7	292.7
Phynylalanine	4.271	628.6	59.8	3408.0
Threonine	4.278	432.5	59.9	2460.9
Tryptophan	-	-	-	-
Valine	6.701	664.3	93.9	3790.1

Protein nitrogen conversion factor 6.09

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Table 23: Non-essential amino acid content of *Lathyrus* protein concentrate (LPC).

Non-essential amino acids	n mole	R.W. (ng)	N	mg/100 LPC
Aspartate	12.25	1409.5	171.5	7873.3
Alanine	6.83	485.3	95.6	2938.6
Glutamate	16.38	2115.6	229.5	11961.0
Glycine	7.39	421.6	103.5	2680.2
Proline	5.84	567.1	81.8	3246.2
Serine	6.78	590.4	95.0	3441.9
Tyrosine	2.19	357.4	30.7	1916.15
½ cysteine	0.37	38.2	5.2	217.4

Protein nitrogen conversion factor 6.09

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3.5 Digestibility of LPC

The *in vitro* digestibility of LPC was 99% (Table 24) i.e almost universal digestibility. The digestibility of the reference protein casein was 99.9%.

Table 24: *In vitro* digestibility of *Lathyrus* protein concentrate (LPC).

* Used (N.mg)	Sample	Residual nitrogen (mg)	Corrected	Digestibility (%)
	Blank	0.055		
	Enzyme blank	0.084		
28.6	Casein	0.100	0.014	99.95
27.6	LPC	0.363	0.279	99.00

* 200 mg protein

3.6 Food intake and body weights of different groups of rats

The initial body weight. of rats in experimentl. I were about 125 to 130 g. and non-protein group was 153 g. Five groups of rats received different percentage protein diet. (Table 25).

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Table 25 : Grouping of rats according to diet given.

(Experiment-1)

Diet/Group	No of rats	Initial body weight	Type of diet	% of Protein
I	10	153±5.0	Non-protein	-
II	6	126±6.5	Skim-milk protein	18.00
III	6	125.58±4.5	LPC	18.00
IV	6	130.37±2.5	Rice protein	6.23
V	6	129.37±3.0	Rice protein + LPC	5.95+3.0 = 8.95

The average amount of food intake by different groups of rats were nearly same, 11 to 17 g per rat per day, during 6 weeks of feeding. But LPC group consumed comparatively lower quantity of diet (9-13g/rat/day) and that of non- protein group only 4-6 g/rat/day (Table 26).

Table 26 : Mean food intake by different groups of rats. (Experiment-1)

Group	Diet	Week					
		1	2	3	4	5	6
I	Non-protein	6.92*	5.45	5.54	4.41	5.69	6.79
II	Skim-milk protein	10.61	12.71	13.93	14.41	13.91	14.96
III	LPC	10.80	11.68	12.41	9.59	9.97	13.71
IV	Rice protein	11.81	14.24	19.90	16.78	10.85	11.50
V	Rice protein + LPC	11.78	12.54	15.65	13.10	17.72	17.70

*Values are average food intake in g/rat/day.

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Table 27 : Mean protein intake by different groups of rats on weekly basis. (Experiment-1)

Group	Diet	Week					
		1	2	3	4	5	6
I	Non-protein	-	-	-	-	-	-
II	Skim-milk protein	1.91* (± 0.29)	2.29 (±0.36)	2.51 (±0.36)	2.54 (±0.38)	2.50 (±0.37)	2.70 (±0.37)
III	LPC	1.83 (±0.23)	2.10 (±0.21)	2.23 (±0.24)	1.73 (±0.19)	1.79 (±0.19)	2.47 (±0.24)
IV	Rice protein	0.73 (0.11)	0.89 (±0.14)	1.24 (±0.14)	1.12 (±0.14)	0.67 (±0.07)	0.72 (±0.007)
V	Rice protein + LPC	1.05 (±0.14)	1.09 (±0.16)	1.4 (±0.21)	1.17 (±0.16)	1.58 (±0.22)	1.57 (±0.21)

*Values are mean ± SD of protein intake in g/rat/day.

In experiment.2 the percentage of protein for all five diets were same (Table 28). The average food intake for group II, IV, & V was 14 to 20 g/rat/day, and that of group III & group I was 9 to 10 g rat/day, and about 4 to 7 g/rat/day respectively. (Table 29)

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Table 28: Grouping of rats according to diet given.

(Experiment-2)

Diet/ group	No. of rats	Initial body weight (g)	Type of diet	% protein in the diet
I	5	68.2±2.5	Non-protein	-
II	5	71.6±2.4	Skim-milk protein	6.5
III	5	71.26±2.5	LPC	6.5
IV	5	72.52±2.6	Rice protein	6.5
V	5	73.68±2.4	Rice protein + LPC (4:1, w/w)	6.5

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Table 29: Average food intake in g by rats of different groups. (Experiment-2)

Group	Diet	Week			
		1	2	3	4
I	Non-protein	5.72 ± 1.21*	7.24 ± 1.25	5.07 ± 0.78	4.04 ± 0.71
II	Skim-milk protein	19.56 ± 1.44	20.39 ± 2.01	19.51 ± 1.78	18.65 ± 1.5
III	LPC	9.73 ± 1.02	10.35 ± 1.33	9.49 ± 1.21	9.31 ± 1.11
IV	Rice protein	16.04 ± 1.2	17.80 ± 1.45	15.12 ± 1.3	14.94 ± 1.25
V	Rice protein + LPC	18.24 ± 1.07	20.26 ± 1.83	18.17 ± 1.64	16.70 ± 1.51

*Values are mean ± SD of food intake per rat/day.

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Table 30: Mean protein intake of different groups of rats.

(Experiment-2)

Group	Diet	Week			
		1	2	3	4
I	Non-protein	-	-	-	-
II	Skim-milk protein	1.27*	1.32	1.26	1.21
III	LPC	0.63	0.67	0.61	0.61
IV	Rice protein	1.04	1.15	0.98	0.97
V	Rice protein + LPC	1.18	1.31	1.18	1.08

*Values are mean of protein intake in g per rat per day.

3.7 Impact of rice protein + LPC on rat growth

Table 32 represents the final weight gain of different groups of rats after 6 weeks of feeding. The skim-milk protein and rice+LPC group gained nearly the same weight (102 ± 12.4 g and 117.0 ± 14.2 g respectively). The final weight. gain of LPC alone (52 ± 10.3) and rice protein alone (97 ± 10.6) was significantly ($P<0.05$) lower than rice+LPC group (117.0 ± 14.2 g).

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Table 31 : Per week mean weight gain/loss* of different groups of rats (Experiment-1)

Group	Diet	Week					
		1	2	3	4	5	6
I	Non-Protein	-2.13**	-1.0	-1.16	-1.38	-0.33	-1.25
II	Skim-milk protein	+3.78	+3.75	+2.27	+2.23	+.92	+1.72
III	LPC	+1.45	+1.81	+1.18	-.33	+.63	+2.67
IV	Rice protein	+2.04	+3.03	+2.07	+2.01	+2.88	+2.82
V	Rice protein+LPC	+3.01	+3.73	+2.34	+2.57	+2.33	+2.72

*+ = gain in weight, - = loss in weight.

**Values are average weight of respective number of animals.

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Table 32 : Impact of *Lathyrus* protein concentrate (LPC) on growth of rats after feeding for 6 weeks.

(Experiment-1)

Group	Diet	Initial body wt (g)	Final body wt (g)	wt gain/loss (g)
I	Non-Protein	153.0 ± 5.0*	02.25 ± 6.0	-50.75 ± 6.0[a]
II	Skim-milk protein	126.0 ± 6.5	228.83 ± 10.7	+ 102 ± 12.4
III	LPC	125.58 ± 4.5	177.25 ± 8.5	+ 52 ± 10.3
IV	Rice protein	130.37 ± 2.5	227.5 ± 4.0	+ 97.1 ± 10.6
V	Rice protein+LPC	129.37 ± 3.0	246.38 ± 9.3	+ 117.0 ± 14.2

*Each value is the mean ±SD of respective number of animals.

[a]All the differences are significant ($p<0.05$) but differences of Group II & IV are insignificant.

Table 34 (Expt. 2) shows that the final body weight gain of skim-milk protein (71.38 ± 2.0) and rice + LPC (64.02 ± 2.8) was nearly the same. The value of final weight. gain of LPC alone (7.78g ± 1.5) was significantly ($p<0.001$) lower than that of rice + LPC group. The weight gain in the rice protein alone was also lower than in the rice + LPC group.

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Table 33: Comparison of per week mean weight gain/loss* of rats of different groups.

(Experiment-2)

Group	Diet	Week			
		1	2	3	4
I	Non-Protein	-0.83**	-1.0	-1.14	-1.28
II	Skim-milk protein	+2.05 ± 0.35	+3.03 ± 0.42	+2.81 ± 0.40	+2.29 ± 0.35
III	LPC	+0.22 ± 0.07	+0.33 ± 0.1	+0.29 ± 0.1	+0.26 ± 0.07
IV	Rice protein	+1.37 ± 0.21	+2.10 ± 0.28	+1.92 ± 0.3	+1.60 ± 0.21
V	Rice protein + LPC	+1.76 ± 0.21	+2.59 ± 0.38	+2.56 ± 0.3	+2.25 ± 0.28

*+ = Gain in weight, - = Loss in weight.

** Values are mean ± SD of weight gain or loss per day per rat.

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Table 34: Impact of *Lathyrus* protein concentrate (LPC) on rat growth after 4 weeks of feeding.

(Experiment-2)

Group	Diet	Mean inital body wt. (g)	Mean final body wt. (g)	Mean wt. gain/loss** (g)
I	Non-Protein	68.2 (± 2.5)*	38.36 (± 3.25)	-29.84 ± 2.5[a]
II	Skim-milk protein	71.6 (± 2.4)	142.98 (± 4.6)	+71.38 ± 2.0
III	LPC	71.26 (± 2.5)	79.04 (± 3.5)	+7.78
IV	Rice protein	72.52 (± 2.6)	121.42 (± 4.7)	+48.97 ± 2.0
V	Rice protein + LPC	73.68 (± 2.4)	137.70 (± 4.9)	+64.02 ± 2.8

*Values are mean ± SD of 5 animals.

**+ = Gain in weight, - = Loss in weight.

[a]All the differnce are significant ($p<0.05$) but group III highly significant ($p<0.001$).

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3.8 Protein excretion by different groups of rats

Every rat of each group was housed in metabolic cage for 24 hours to collect feces and urin to determine the excreted protein. Table 35 represents the amount of excreted protein of different group of rats. The protein content of non-protein group through feces and urine was 0.038 g/rat/day and 0.083 g/rat/day respectively.

In 1st week (Expt. 1) the amount of excreted protein of group II was 0.15 g/rat/day and 0.18 g/rat/day through feces and urine respectively and that of group V was 0.16 g/rat/day and 0.25g through feces and urine respectively.

In 2nd week as shown in Table 35 the both groups excreted nearly the same amount of protein through feces and urine (0.07 g and 0.42/0.12 and 0.40g) respectively. The excreted protein for group III and group IV was 0.11g (feces), 1.02g (urine), and 0.06 (feces) and 0.42 g (urine) respectively.

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Table 35 : Mean protein excretion in feces and urine.

(Experiment-1)

Group	Week					
	1		2		5	
	feces	urine	feces	urine	feces	urine
I	0.038*	0.083*	0.020	0.063	tr**	tr
II	0.15	0.48	0.07	0.42	0.27	0.80
III	0.11	1.02	0.08	0.50	0.14	0.59
IV	0.063	0.425	0.05	0.43	0.05	0.45
V	0.16	0.25	0.12	0.40	0.20	0.66

*Values are average protein excretion in g/rat/day.

**tr = Trace.

As is shown in Table 36 (Expt - 2). the excreted protein (0.07 g, 0.28g) for group II and that of group V (0.06 g, 0.31 g) was almost same. The excreted protein for group III was 0.06 g/rat/day and 0.36 g/rat/day and that of Gr. IV was 0.12 g and 0.47 g.

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Table 36: Mean protein excreted through feces and urine.
(Experiment-2)

Group	Diet	Week			
		1		2	
		Feces	Urine	Feces	Urine
I	Non-protein	0.038*	0.083*	0.035	0.078
II	Skim-milk protein	0.07	0.28	0.09	0.35
III	LPC	0.06	0.36	0.06	0.38
IV	Rice protein	0.12	0.47	0.09	0.45
V	Rice protein + LPC	0.06	0.31	0.05	0.32

*Values are average excretion of protein in g/rat/day.

3.9 Impact of rice protein + LPC on protein efficiency ratio (PER)

Table 37 (Expt. 1) represents the effect of rice protein + LPC on PER. The PER value of rice protein + LPC (2.5) was significantly higher that that of either LPC alone (1.30) or rice alone (2.29).

Table 38 (Expt-2) also showed that the PER value of skim-milk protein (2.01) and that of rice + LPC (1.92) were almost same. The LPC diet showed significantly ($P<0.01$) lower PER value (0.45) than all other diets.

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Table 37: Increased protein efficiency ratio (PER) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice (Experiment-1)

Group	Diet	Initial body wt. (g)	Final body wt. (g)	Weight gain (g)	Protein intake (g)	PER*
I	Non-Protein	-	-	-	-	-
II	Skim-milk protein	126.01** (± 6.5)	210.36 (± 8.8)	84.36 (± 10.5)	65.09 (± 10.12)	1.30 ± 0.01[a]
III	LPC	125.58 (± 4.5)	154.47 (± 7.0)	28.89 (± 8.5)	55.28 (± 7.5)	0.52 ± 0.00
IV	Rice protein	130.37 (± 2.5)	192.0 (± 3.5)	64.1 (± 10.6)	28.00 (± 5.1)	2.29 ± 0.02
V	Rice protein+LPC	129.37 (± 3.0)	211.0 (± 7.8)	81.63 (± 12.4)	33.06 (± 6.5)	2.50 ± 0.00

*Values are obtained after 28 days of feeding

**Values are mean ± SD of respective number of animals.

[a]The differnce bet[n] Group IV & V are not significant. But all other values are significantly ($p<0.01$) different.

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Table 38: Protein efficiency ratio (PER) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice.

(Experiment-2)

Group	Diet[1]	Intial body weight (g)	Weight gain (g)	Food intake (g)	Protein intake (g)	PER[2]
I	Non-protein	68.2*±2.5	-	-	-	-
II	Skim-milk protein	71.6±2.4	71.38±3.5	546.9±10.2	35.5±6.2	2.01 ± 0.01[a]
III	LPC	71.26±2.5	7.78±3.0	272.3±6.5	17.7±5.1	0.45 ± 0.001
IV	Rice protein	72.52±2.6	48.97±4.8	447.7±9.2	29.1±5.9	1.68 ± 0.05
V	Rice protein + LPC	73.68±2.4	64.02±4.9	512.8±9.8	33.4±6.0	1.92 ± 0.03

[1]Total protein percent in diets 2 to 5 was 6.5%. In group V rice protein was 5.2% and LPC was 1.3%.

[2]Values obtained after 28 days of feeding.

*Values are mean ± SD of 5 animals.

[a]All the differences are highly significant ($p<0.01$) except group II & group V.

3.10 Impact of rice protein + LPC on biological value (BV)

The results obtained from the BV determination have been shown in Table 39 and Table 40 for Expt. 1 and Expt. 2 respectively. The LPC alone and rice protein alone showed significantly ($P<0.01$) lower BV value than other diets. The BV value of skim-milk protein (78) & that of rice protein + LPC (82 ± 1.30) were nearly the same and significalnly higher than in other groups. Similar results were obtained in Expt. 2 (Table 40).

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Table 39: Increased biological value (BV) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice.

(Experiment-1)

Group	Diet	N intake (g)	N absorbed (g)	N retained (g)	BV*
I	Non-Protein	-	-	-	-
II	Skim-milk protein	1.91**	1.798	1.401	78 ± 1.20[a]
III	LPC	1.83	1.758	0.821	47 ± 0.10
IV	Rice protein	0.815	0.790	0.448	57 ± 0.50
V	Rice protein+LPC	1.05	0.928	0.761	82 ± 1.30

*Values obtained after 10 days of feeding.

**Values are average intake of nitrogen per rat per day.

[a]All the values are significantly ($p<0.01$) different.

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Table 40: Biological value (BV) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice protein.

(Experiment-2)

Group	Diet	N intake[1] (g)	N absorbed (g)	N-retained (g)	BV[2]
I	Non-Protein	-	-	-	-
II	Skim-milk protein	1.27	1.238	1.041	84 ± 1.22[a]
III	LPC	0.632	0.61	0.333	55 ± 0.80
IV	Rice protein	1.04	0.98	0.573	58 ± 0.50
V	Rice protein + LPC	1.19	1.168	0.941	81 ± 1.00

[1]Values are the mean of 5 animals.

[2]Values obtained after 7 days of feeding.

[a]All the differences are significant ($p<0.01$) except group II & V.

3.11 Impact of rice + LPC on NPU value

Table 41 (Expt-1) shows the NPU values of different types of diet. The LPC and rice protein diet alone showed significantly ($P<0.01$) lower value than that of other diets. The NPU value of rice + LPC was increased to the value 72 ± 1.50 which was almost the same as the NPU value of skim-milk protein (73 ± 0.88). Similar results were obtained in Expt. 2 (Table 42)

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Table 41: Increased net protein utilization (NPU) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice. (Experiment-1)

Group	Diet	Nitrogen intake (g)	Nitrogen retained (g)	NPU*
I	Non-Protein	-	-	-
II	Skim-milk protein	1.91**	1.401	73 ± 0.88[a]
III	LPC	1.83	0.821	45 ± 0.50
IV	Rice protein	0.815	0.448	55 ± 0.70
V	Rice protein+LPC	1.05	0.761	72 ± 1.50

*Values obtained after 10 days of feeding

**Values are average intake of nitrogen per day.

[a]All the differences are significant ($p<0.01$) except group II & IV.

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Table 42: Net protein utilization (NPU) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice.

(Experiment-2)

Group	Diet	N intake[1] (g)	N retained (g)	NPU[2]
I	Non-protein	-	-	-
II	Skim-milk protein	1.27	1.041	82 ± 0.70[c]
III	LPC	0.632	0.333	53 ± 0.89
IV	Rice protein	1.04	0.573	55 ± 0.25
V	Rice protein + LPC	1.19	0.941	79 ± 1.30

[1]Values are the mean of 5 animals

[2]Values obtained after one week of feeding.

[c]All the differences are highly significant ($p<0.01$) but that of between group II & V is insignificant.

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Table 43: Protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU) of *Lathyrus* protein concentrate (LPC) when fed mixed with rice.

(Experiment-1)

Group	Diet[1]	PER[2]	BV[3]	NPU[3]
I	Non-Protein	-	-	-
II	Skim-milk protein	1.30[4]	78	73
III	LPC	0.52	47	45
IV	Rice protein	2.29	57	55
V	Rice protein + LPC[5]	2.5	82	72

[1]Total Protein Percent in diet II and III 18% and diet IV and V 6.23% and 8.95%

[2]Values obtained after 4 weeks of feeding

[3]Value obtained after 1 week of feeding

[4]Each value is the mean of respective no. of animals

[5]Rice protein 5.95%, LPC 3.0%, totally 8.95%.

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Table 44: Protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU) values of *Lathyrus* protein concentrate (LPC) fed alone or mixed with rice.

(Experiment-2)

Group	Diet[1]	PER[2]	BV[3]	NPU[3]
I	Non-protein	-	-	-
II	Skim-milk	2.01[4]	84	82
III	LPC	0.45	55	53
IV	Rice	1.68	58	55
V	Rice + LPC[5]	1.92	81	79

[1]Total protein percent in diets of group II to V was each 6.5%

[2]Values obtained after 4 weeks of feeding

[3]Values obtained after 1 week of feeding

[4]Values are mean of 5 animals

[5]Rice protein, 5.2% plus *Lathyrus* protein concentrate, 1.3% totaling 6.5%

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3.12 Effect of LPC on serum lipid profile and total protein

Table 45 represents the effects of LPC diet on serum total cholesterol, HDL, LDL, TG and serum-total protein in relation to other diets. The cholesterol level of LPC diet (63.5 ± 5) was significantly (P<0.05) lower than that of skim milk protein diet group (80.7 ± 12.2). The other diets groups also showed significantly lower value than skim milk protein diet group. The HDL value of all diets group were almost same except the non-protein group; it showed significantly lower value. The LDL values of all diet groups also showed same value except the non-protein group . The TG value of skim-milk protein group showed higher value than that of LPC diet group. The level of total protein of all groups were almost same. So, LPC seemed to have positive effects on serum lipid profile. The reason is not clear at this time but higher fibre content in the LPC diet weight be responsible for this.

Table 45: Effect of *Lathyrus* protein concentrate (LPC) on serum lipid profile and total protein.

Group	Diet	Serum total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Triglyceride (mg/dl)	Serum total protein (g/dl)
I	Non-protein	45.1±8.0^{c}	15.05±16^{b}	38.29±3.5^{b}	34.03±7.32^{c}	4.65±0.60^{b}
II	Skim-milk protein	80.7±12.2^{a}	34.70±3.9^{a}	49.52±6.1^{a}	155.9±33.9^{a}	6.0±0.34^{a}
III	LPC	63.5±5.0^{b}	36.9±5.2^{a}	47.2±5.0^{a}	81.4±28.5^{a}	6.4±0.6^{a}
IV	Rice protein	60.5±1.52^{b}	35.1±3.5^{a}	41.52±5.0^{a}	94.9±29.1^{a}	6.1±0.72^{a}
V	Rice protein + LPC	62.7±5.6^{b}	32.2±3.1^{a}	50.28±4.5^{a}	62.1±5.1^{b}	6.9±0.5^{a}

Values with different superscripts are significantly different (P<0.05), values are mean ±SD of 5 animals.

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Chapter 4

DISCUSSION AND CONCLUSION

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4. DISCUSSION & CONCLUSION

4.1 Discussion

The nature and extent of the protein problem in Bangladesh have been studied by many investigators. It is now the consensus of the experts that the problem is essentially a protein calorie problem (FAO/WHO,1970), particularly for the growing children. The protein requirement of this vulnerable group is higher than that of the other population groups. Failure to meet the protein energy need during growing age is liable to have serious consequences in later life. This is the underlying cause of high child mortality rate and its irreversible damage to body tissues, including brain growth and function (Yusuf, 1992).

The main cause of the protein problem in our country is dietary calorie dificiency (10-20%) (SNB, 1995) and also dependence on food stuffs with low protein content with low biological value. The methods of child feeding are also unsatisfactory. Exclusive breast feeding (only breast milk up to 5-6 months of age) is almost non-existent. Also, weaning is very poor, being insufficient to meet the need of the growing child when it enters a critical period of life.

The protein problem is also acute in many other countries of the world. To combat this, suggestions have been made by FAO, WHO, UNICEF and many other organizations to use the local cheap protein sources for preparation of protein-rich food for the growing children.

Bangladesh produces about 500,000 MT of pulses (BBS, 1994). Some other varieties are also being introduced with high expectations. These pulses may play an important role in meeting our protein need. However, unfortunate though it is,

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the daily percapita intake of pulses is only 10-12 g (BBS 1994) compared to around 40g/person/day in India (Kumar *et al.*, 1991)

Of the pulses produced, Khesari constitutes about one third of total production. It is the cheapest (current market price, Taka 20/kg, compared to Tk. 30-40/kg for other pulses). We therefore decided to introduce Khesari as a cheap and locally available protein source for a protein concentrate preparation. Khesari is not only cheap, it is also highly nutritious, containing 28-30% protein. It can be cultivated in diverse ecological condition such as drought, flood, saline water. It needs no care as in other crops. It therefore occupies an important position in the cropping pattern of Bangladesh.

The present investigations, both chemical and biological, indicate that *Lathyrus* protein concentrate (LPC) has good nutritive value and is better than those of many other single vegetables.

De *et al.* (1966) have made some studies on the nutritive value of some protein rich food preparations using cereals legumes and fish protein concentrate (FPC).

The protein extraction procedure followed in the present study was very simple and easy; only two steps were involved, and the protein recovery was 66% which contain 90% protein and virtually free of toxin (Tables 18 & 19).

Khaleque and Rahman (1982) found 69% extracted protein from Khesari. But they did not find out its toxin content.

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The toxin was removed after acid wash. Finally the LPC contained 0.0012% toxin. (Table-20), which shows the LPC to be virtually free of the toxin and safe for human consumption (see later).

According to the research made in India and other countries it was believed that *Lathyrus sativus* with less than 0.2% ODAP is to be considered a low toxin variety, while variety with more than 0.42% ODAP should be considered high toxin variety (Zhong, 1995).

Naved *et al.* (1990) also reported that simple steeping of Khesari seed and then discarding the water would eliminate 80% of the free ODAP.

Lambein *et al.* (1995) reported that double 48 h fermentation of *Lathyrus* seed with *A. oryzae* NRRL and *R. obligosporus* reduced more than 90% of neurotoxin ODAP.

The protein content of the final LPC in the present study was about 90%. LPC thus was a good food protein concentrate.

The LPC was found 99% digestible (Table-10) as determined by *in vitro* treatment with pepsin and pancreatin. The digestibility of the reference protein casein determined in the same run was 99.9%. The almost absolute digestibility of LPC presents itself as an ideal food protein concentrate for dietary use for people of all ages, particularly small children.

The proteins of the LPC were separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. The LPC separated into 4 major fractions of apparent molecular weight 113,000, 100,000, 55,000 and 24,000 respectively

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(Fig. 3) All these protein fractions belong to the globulin family (legumin, vicilin, convicilin), as is found in other pulses (Guldager, 1978; Croy *et al.*, 1980). The electrophoretic pattern thus shows that there occurred no internal polymerization of the *Lathyrus* protein components during the preparation of LPC. The almost universal digestibility (see above) also indicates absence of polymerized protein which are rather difficult to digest.

As expected, the contents of the sulphur amino acids cysteine and methionine in LPC were rather low, only 217 to 293 mg % (Table-21). All other amino acids were present in appreciable quantities. Chowdhury (1988), Low *et al.* (1990) and Rotter *et al.* (1991) also have shown that *Lathyrus* seed contained adequate quantity of all the amino acids, particularly lysine but deficient in methionine and cysteine. The moderately high quantity of lysine (4.06 g %) (Table 22) is particularly interesting, because this amino acids is the limiting amino acid in rice. Rice protein, on the other hand, is rich in methionine and cysteine. Therefore a mixture of rice and LPC should form a mutually supplemented good quality protein diet.

Low *et al.* (1990) suggested that supplementation of *Lathyrus* with methionine alone on combination with tryptophan would be helpful in improving feeding value.

Chowdhury (1995) showed an alternative way to balance the methionine deficiency of *Lathyrus sativus* seeds by other plants protein, for example, sesame oil meal which is rich in methionine and is available in Bangladesh at a reasonable cost.

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To investigate the nutritive value of LPC two experiments were done. In one experiment, the percentage protein in different groups was variable; in the reference group (skim milk), protein was 18% whereas in rice + LPC groups it was around 9% (Table-25). In the other experiment, the protein percentage was kept the same (around 6.5%) in all groups (Table-28).

This was because the maximum value of rice protein was between 6 to 7%. So we had to maintain this level in all the experimental diet to make valid comparison of growth in the different groups.

In both experiments each diet showed almost the similar growth ability on rats. Although in the first experiment the skim-milk protein content was 18%, the rats showed poor growth (Tables 31,32). It might be due to the watery loose motion of the rats, probably due to indigestion of the high protein.

In experiment I the mean weight reduction in the non-protein group was 50.75 g; the mean weight gains of group II (skim milk), group III (LPC), group IV (rice), and group V (rice + LPC) were 102 ± 12-4, 52±10.3, 97.1±10.6 and 117.0±14.2 of respectively (Table-32).

Thus, rice + LPC diet showed higher growth than LPC alone or rice protein diet alone.

Similarly, in the other experiment LPC alone showed a rather poor growth promoting ability but when mixed with rice (rice protein + LPC = 4:1 w/w), the ability increased dramatically to almost that of skim-milk (Tables 33, 34).

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Rice protein alone also had a lower ability than when supplemented with LPC. This confirms mutual supplementation of rice protein and *Lathyrus* protein.

Bressani and Rodolto (1963) reported that pumpkin flour protein at both 10 and 15% protein level induced better growth in the adult protein depleted rats than did skim-milk. Pumpkin flour with more than 35% of the diet did not result in further growth in rats. The protein efficiency ratio decreased as the protein percentage of the diet increased.

Pumpkin is apparently a good source of lysine but deficient in methionine and threonine. Their report indicated that supplementing pumpkin flour with either threonine or methionine alone did not improve growth. Addition of the two amino acids, however, increased growth and improved both feed and protein efficiency.

The protein efficieny ratio (PER) of LPC, when fed alone was very low, 0.45 compared to 1.68 of rice protein. But when LPC was fed mixed with rice in the ratio of 4:1 w/w the PER value increased to 1.92 which was very close to the PER value of skim-milk protein diet, 2.01 (Table-38). In the case of experiment I the LPC alone showed PER of 0.52, but when LPC was fed mixed with rice protein (2:1 w/w) the PER value increased to 2.47.

Kabirullah *et al.* (1976) constituted different experimental diets of different sources of plants and animal protein. From their investigation it was found that diet containing Bengal gram, rice, wheat, groundnut, and fish protein concentrate (FPC) showed higher PER value than all other diets which contained higher amounts of FPC. PER value was close to skim-milk diet.

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Similarly the biological value of LPC alone in the present experiment was 55 and that of rice alone was 58. But the BV of rice + LPC was 81, close to the BV of skim-milk, 84 (Table 40). Experiment I also showed similar effect (Table-39).

Likewise the NPU of LPC alone was only 53 but when mixed with rice, the value increased to 79 near the value of 82 of skim-milk protein diet (Table-44). So, LPC showed growth promoting ability in all respects of measurable protein quality indicators.

These findings in rats are supported by the results of a recent pilot study in which LPC was fed to young children aged 1-3 years. In this study, young babies were given 10g of LPC as a protein supplement every day for 3½ months. In the control group, age matched children were given all other food ingredients except LPC. After 3½ months, the body weight gain in the LPC-fed children was significantly higher compared to that in the control children (Ahmed and Yusuf, 1995).

On the other hand LPC also had influence on lipid metabolism as described in Table-45. Blood cholesterol level was lower in rats which received LPC than those which received skim-milk protein. This is an additional area of further research. One reason may be due to high fibre content in the LPC diet; fibre has been shown to lower blood cholesterol level (Kawatra *et al.*, 1991).

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4.2 Conclusion

Protein concentrate from *Lathyrus sativus* has been prepared which is easily digestible and which supplements cereal protein (rice protein) favourably to make the mixture as growth promoter potential in both rat and human. It can be used to supplement cereal-based food. The LPC thus appears very promising in the context of protein energy malnutrition in our country.

A great deal can now be done with the LPC. Attempts should be made for large scale preparation of LPC. A feasible study may be undertaken for distribution of small packets of LPC (say in 20g) all over the country for human consumption after being cooked with rice (not discarding water) or with vegetable. A pilot study is now under preparation.

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Chapter 5

REFERENCES

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5. REFERENCES

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