A NEW NUTRITIONAL IDEA FOR MAN:
LUCERNE LEAF CONCENTRATE

Dr. V. ZANIN, October 1998

In collaboration with:
Pr. Ph. BOUCHET (UFR de Pharmacie de REIMS), Pr. H. CHOISY (CHU de REIMS), Pr. J.C. DILLON (Institut des Cordeliers de PARIS), de Mrs E. HENNEQUIN (CAVISA), du Dr. E. KARIGER (CHU de REIMS), du Dr. C. RECCHIA (Nutritionist), de Mr Eric SEILER (FRANCE LUZERNE), de Mr. B. THAREL (Plant Selection).

And:
Mrs I. GASTINEAU, Mr. R. DOUILLARD, Mr. O. de MATHAN, Mr. J.M. MOJON, APEF members
and Mr J. SUBTIL, APEF President

(English Translation by M.N.G. DAVYS, formerly of Rothamsted Experimental Station)
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PROLOGUE
Jacques SUBTIL (Chairman, APEF)

In the late 1970’s, some officers of the agricultural co-operative FRANCE LUZERNE (which specializes in forage drying) realized that one of their products, a concentrate made from lucerne leaves, could be suitable for human consumption, given some modification of the extraction process.

In 1993, they formed « l’Association pour la Promotion des Extraits Foliaires en Nutrition (APEF), a non-profit association, in order to study, put into practice, evaluate and develop this innovation. It consists of extracting the most valuable nutritive components (proteins, vitamins & trace elements) of lucerne or other green vegetation and then using them as a dried, digestible concentrate to supplement the basic diet of malnourished people.

Several feeding trials of lucerne leaf concentrate used in this way (for children, from 6 months-6 years old; pregnant and lactating women; the elderly) have been successfully carried out in Romania, China and Nicaragua.

The first results confirm the nutritional worth of this concentrate: used at low dosage (6-10 g/day) it effectively corrects dietary deficiencies, improves individuals' health and children's development.

At the same time, it helps avert certain diseases associated with malnutrition, in particular anaemia, diarrhoea, respiratory infections and nutritional blindness (xerophthalmia).

We knew, too, that an English Charity (Find Your Feet) had pioneered, in several countries of Asia and Latin America, centres of protein extraction from local green vegetation in simplified form. In spite of their crudity, their products had already brought very satisfactory results reported in several medical journals.

This new idea for a nutritional supplement seems more and more to be one which might supply an efficacious long-term solution to the dietary imbalance for families and populations in difficulty. Thanks to their richness, notably in lysine, tryptophan, iron, calcium and vitamins, leaf concentrates restore the balance of diets based on seeds. They improve growth and resistance to disease. In very young children they allow normal development of the brain and thus safeguard the ability to benefit from education and so improve the chance of a better life.

Moreover, they are an alternative to those foods of animal origin or of the fruits and vegetables generally missing from the diets of the impoverished for they are:

- Relatively easy to make, distribute and use,
- Very cheap: 30 FF/children/year (price in France),
- Of exceptional nutritive value,
- Reproducible world-wide and
- Dependant only on the enormous and inexhaustible supplies of leaf.

Leaf concentrate thus shows itself to be a novel food of great significance to man.

It merits special attention and official recognition in the medical world and by the Ministry of Food.
INTRODUCTION

In tribute to the late Professor Henri LESTRADET, a strong supporter of the project, who wrote, shortly before his death, the following:

« More than half the world’s population sees its health, even its survival, threatened by serious nutritional deficiencies:

- Protein deficiency, with or without calory deficiency, at the root of problems of growth and immunity,
- Vitamin A deficiency affects more than 10 million children, causing half the blindness in the world. Hypovitaminosis A is defined by WHO as the second priority after protein and caloric malnutrition,
- Iron deficiency, even more widespread, since according to UNICEF 500 million children suffer chronic anaemia with its accompanying immunity problems and hindrance to physical and mental growth.

For decades, WHO has had a programme for fighting these 3 deficiencies but while the need is great the cost is high. Food Aid, which can come only from rich countries, can never supply all that is needed. Moreover it can be but a palliative, for the final solution lies, of course, in nutritional self sufficiency.

Now, for several years English researchers have investigated a new way of producing protein, vitamin A, iron and calcium. This is from vegetable sources as yet unexploited but present in enormous quantity where rainfall is sufficient, namely the leaves of plants which man has not hitherto known how to turn to good account.

In fact, only after an extraction that releases from the cellulosic membrane the protein, vitamin A and iron (plus calcium, magnesium and folic acid) do they become easily absorbed by the human digestive system. The necessary extraction can certainly be done industrially at relatively low cost, but it can equally be done with simple equipment that is easy, at least in theory, to set up.

Preliminary studies have been made with apparently interesting results. These need, of course, to be followed up scientifically, looking at the problems of the acceptability of these leaf products (industrial or artisanal) with regard for culinary and cultural customs, especially with children in mind.

In any case now that the technology exists and the first results are promising, it seems urgent to undertake well structured applied research, carried out methodically and with determination, but also with enthusiasm for the nutritional stakes are considerable.»

Professor Henri LESTRADET, Feb. 1996
Formerly member of National Academy of Medicine and President of the Nutrition Society of France.
I – LUCERNE

« Lucerne is the marvel of the fields »
Théâtre de l’Agriculture et Mesnage des Champs
Olivier de Serre  1539-1619  (Gardener to the King)

1. Origin and genetics

1.1. History

Lucerne was introduced into Europe about 470 BC during the Persian war. It was then called Medica Herba « Herb of Media », which later become the generic name Medicago.

However, Hittite tablets had already recorded its use as winter feed for animals around 1 300 BC. It came originally from Caucasian lands of Iran and Turkey where it was known as alfalfa « the best forage ».

Lucerne appeared in France in the 16th century but it was only in the 18th century that it became widely known when it was shown to be an improvement on fallowing and that it enriched the soil with nitrogen.

Nowadays, Lucerne is the forage most widely cultivated in the world. It is particularly distributed in warm temperate and subtropical zones and at altitude (Mauriès, 1994).

1.2. Genetics of Lucerne

Lucerne is a legume capable of fixing atmospheric nitrogen thanks to a symbiosis established with a bacterium within its root system.

1.2.1. Species

The name lucerne covers two botanical species and their hybrids (Mauriès, 1994) :

* Medicago sativa L., characterised by taproots able to reach a depth of up to 10 metres : very well adapted to hot climates,

* Medicago falcata L., highly frost resistant and with low nutritional demands,

* Varia (Hybrids).

1.2.2. Varieties cultivated in France

In France, all the populations of Lucerne consist of Medicago Sativa or of genetically close hybrids (varia) (Mauriès, 1994), eg. :

* Provence : essentially Medicago sativa,

* Flamand : mixture of Medicago sativa with a high proportion of hybrids used for crop-drying in the cooler regions.

2. Culture of Lucerne

France is one of the two greatest producers of lucerne in Europe. It uses, almost exclusively, improved varieties of the type « Flamand », resistant to Verticillium wilt, a disease often causing serious loss of yield.

2.1. Environmental requirements

* Soil : Lucerne needs a lot of calcium. Optimally it should grow in calcareous healthy soil with a pH between 6 and 7.5. Per tonne of dry matter (DM) produced it will export 30 kg K, 9 kg P and 3 kg Mg. In an average soil only K needs to be supplied. Adding nitrogen is pointless because of lucerne’s ability to use atmospheric nitrogen and the mineral nitrogen in the soil. Its excellent root system allows it to draw up and make thorough use of the soil nutrients.

* Temperature : Optimal growth is had between 15 and 30° C.
Water: Lucerne grows well in zones of well distributed rainfall: shortage of water greatly slows the development of shoots; an excess encourages fungal attacks and deprives the roots of oxygen.

Light: In conditions otherwise non limiting, growth depends directly on visible radiation received.

2.2. Growth
Lucerne is a perennial lasting 2 – 10 years according to its treatment. It regrows after winter or after each cut with the aid of the reserves accumulated in its roots during vegetative growth which lasts up to 10 months in California or Egypt where 8 – 10 cuts /year ar taken on irrigated lands. In Europe, it grows from March to October with, on average, cuts at intervals of 35 – 45 days according to the temperature. It withstands frost very well; in cold spells it is dormant. In Spring it sprouts tillers from its main stalk.

2.3. Treatments
These are extremely limited and are carried out in accordance with good agricultural practice, as promulgated by specialist bodies, most importantly the French National Union of Crop-Dryers (time of application,approved products & dose rates, etc.).

Herbicide: One treatment per year and that only in case of an invasion) is applied to kill self propagating weeds. It is applied at the beginning of winter to ensure that rain will remove all toxic residues in the legumes whose regrowth starts in March.

Insecticide: is used only in the rare event of an attack by weevils. As with all treatments, it is applied at least 3 weeks before a harvest. Actually, the frequency of cutting itself reduces the risk of contamination. In practice, chemical intervention occurs only in an emergency once every 3 to 5 years.

It is worth remarking that those frequent harvests, as well as limiting insect attack, also restrict weed growth and, to a lesser extent, the development of moulds. On the other hand, enormous progress has been made through the selection of varieties resistant to various attacks (verticillium wilt, nematodes…).

Seed treatment: This is undertaken only when necessary to control nematodes with methyl bromide.

2.4. Environmental impact of lucerne culture
2.4.1. Lucerne as an ecological plant
Lucerne is one of the most productive plants in tons of protein per hectare. It mobilises a great deal of nitrogen: N content (as % DM) of the roots is 1.8 and of the above-ground plant 3.5.

Lucerne fixes atmospheric nitrogen but uses preferentially any nitrate N present in the soil: the concentration of nitrate N in the soil diminishes year upon year under lucerne, as the plant recovers it, so diminishing the leaching of surplus nitrate and thus protecting the ground water (Muller et al., 1989).

2.4.2. Mineralization
Nitrogen released by the roots can be used to fertilize following crops. Lucerne is therefore an ecologically valuable plant which plays an important role in the environment (Mullet et al., 1989).

3. Harvest and conservation of Lucerne
Lucerne can be used in several ways:

By forage harvesting: for animal feeding on fresh cut crop.

As pasture: for extensive grazing. The main disadvantages are in the damage to young growth through trampling and uprooting and in the risks of bloat in the ruminants.

As silage: anaerobic conservation by rapid creation in a silo of sufficient acidity to inhibit putrefying flora, it requires technical expertise and considerable equipment.
Hay: is by far the most common use, however it demands favourable weather: rain will lower the quality of the harvest and can even cause its total loss. Moreover, there is often significant loss of leaf during recovery.

Haylage and baling: recently developed methods of anaerobic conservation.

These different methods all suffer the disadvantage of the loss of a good deal of nutrients. That is why a new method of conservation by artificial «industrial» drying was developed in France after the war. This technique consists of drying the lucerne immediately after harvest in a rotating drum at high temperature (700/800° C) followed by storage in an inert atmosphere to protect the more oxidisable components, particularly the pigments and vitamins.

This method of drying has numerous advantages:

- It improves the return to the grower by avoiding the losses inherent in the other methods,
- It allows the taking of regular cuts without being subject to the weather and extends the productive season,
- It simplifies the incorporation of Lucerne into modern stock-feed compounds,
- It permits very good preservation of the nutritive quality of Lucerne, particularly in vitamins and carotene, due to its treatment immediately after cutting. The phenomenon of the tanning of the proteins by the heat partly protects them from degradation in the rumen and improves the digestibility of protein ingested by ruminants,
- It reduces the risk of mould and degradation in storage.

Appearing in France in the fifties, Lucerne drying has seen considerable growth recently. At present in France it involves 110 000 hectares, of which 80% is in Champagne Ardenne (production of about 12 to 15 tons DM/ha/year). This is marketed as a range of preparations growing ever wider (pellets large and small, more or less rich in protein, fibre and carotene; long fibred dried lucerne…) directed at a large assortment of animals (hens, rabbits, sheep, pigs, cattle, horses…).

With the aim of increasing the nutritive potential of lucerne in animal feeding, the Union of Coopératives «FRANCE LUZERNE» located in Champagne Ardenne had the idea, in 1970, to introduce a process of extraction before the drying. In 1975, after 3 years of studies, research and the setting up of an experimental pilot plant, the first industrial plant was installed. Now only three factories of the type in the world use the process. They produce 12 000 T of dried Lucerne concentrate as a by-product from the pressed fibrous residue containing 92% of the original DM, i.e. about 140 000 T for cattle and rabbits.

II – LUZERNE CONCENTRATE

Lucerne is, among the legumes, the plant with the highest yield of crude protein/ha: 2 000 – 3 000 kg, i.e. 3 times more than soya, twice that of peas and 4 times more than wheat. But because of its content of cellulose, hemicellulose and lignin, fibres scarcely digestible by monogastrics, dried lucerne is unsuitable for compounds for certain young «high performance» animals (hens, chicken, calves, piglets). This is why FRANCE LUZERNE went in for a concentrated protineaceous extract of lucerne before the traditional drying process.

The principle lies in heat coagulation of the proteins (precipitation of protein at high temperature, Scheme I page 9. The product has a cellulose content reduced to 1 – 2 %, it is rich in protein (50 – 60 %), in trace elements, vitamins, xanthophylls and carotenoid pigments (Gastineau et de Mathan, 1981).
Block diagram: Process of fractionation of fresh lucerne yielding lucerne leaf concentrate
[Example based on 500 kg of a crop having 20 % dry matter (DM) as harvested: all figures in kg]

**Village process**

- **HARVEST**
- **WASH**
- **PULP**
- **PRESS**
- **GREEN JUICE**
- **HEAT**
- **FILTER**
- **PRESS**
- **BROWN JUICE** or «WHEY»
  - DM: 13
  - MOISTURE: 151
  - TOTAL: 164
- **MOIST CAKE** of leaf concentrate
  - DM: 5
  - MOISTURE: 5
  - TOTAL: 10
- **CP:** 2 – 2.5
- **FINAL DRYING** (OPTIONAL)

**Industrial process**

- **HARVEST**
- **PULP**
- **PRESS**
- **GREEN JUICE**
  - DM: 23
  - MOISTURE: 200
  - TOTAL: 223
- **PREHEAT**
- **STEAM INJECTION** (14)
- **CENTRIFUGE**
- **BROWN JUICE** or «WHEY»
  - DM: 15
  - MOISTURE: 206
  - TOTAL: 221
- **COAGULUM** or CURD
  - DM: 8
  - MOISTURE: 8
  - TOTAL: 16
- **DRY**
- **DRY LEAF CONCENTRATE**
  - DM: 8
  - MOISTURE: 0.6
  - TOTAL: 8.6

**DM:**
- 100
- 23
- 100
- 77
- 13
- 5
- 10

**MOISTURE:**
- 400
- 200
- 5
- 151
- 5
- 5
- 0.6

**CP:**
- 4 – 4.5

*CP (Crude Protein) Total N x 6.25*
1. **Industrial process** (Shematic diag.)

1.1. **Pulping and pressing of lucerne**

Immediately after cutting, the lucerne is pulped and pressed forcefully to separate a large part of the nutritional factors from the indigestible fibre. Done rapidly, this stage also limits hydrolysis of cellular proteins by proteases. The nutritional elements, consisting principally of chloroplastic and cytoplasmic proteins, pigments and vitamins, are recovered in the green juice expressed.

The co-product of this green juice, the ligneous and cellulosic bits of the stalk and leaves, is dried and used as fodder, it still contains plenty of nutrients. Its overall nutritive value, moreover, is improved because of the shattering of the stalk fibres (the nutrients are freed and the fibres themselves more easily digested by the cellulytic ferment in the rumen).

1.2. **Heat coagulation of protein**

The green juice, adjusted to pH 8.5 to slow down the action of phenyloxydase and to improve the struture of the coagulum, is pre-heated and then brought to 85-90°C by steam injection. This causes coagulation of almost all the proteins with which are entrained the pigments, fat-soluble vitamins, lipids and minerals.

1.3. **Separation of the coagulum**

Next, the coagulum (moist green curd containing the majority of the nutrients) is centrifuged from the rest of the solution (brown juice).

This curd contains more than 50 % of crude protein of which 80 % is true protein, accompanied by some free amino-acids or peptides. Heat coagulation extracts on average 8 % of the original DM of the crop and 20 – 25 % of its protein.

1.4. **Drying and storage**

The curd, as paste, having been mechanically separated from most of the brown juice is dried on a fluidised bed. So far, the making of concentrates for animal or human consumption is the same.

Then, the PX Super (commercial name of the concentrate destined for animal feed) is pelleted and stored in sealed silos' cells under inert gas, awaiting distribution.

For human feeding, the product called « Extrait foliaire » (Leaf concentrate) is crumbled. The crumbs (on average with moisture content of 8 %) must be kept dry, away from air, heat and light. Thus, they are bagged hermetically immediately.

To protect pigments and vitamins an antioxidant is added :

- For animal feed : Ethoxyquin (150 mg/kg)
- For human feed : Ascorbic acid (500 mg/kg).

1.5. **Co-product usage**

The brown juice, still containing 13 – 15 % of the original DM (soluble N, mineral salts and sugars, mainly) is mixed with the fibrous residue. The mixture is dried in high temperature air in a rotating drum. The co-product thus obtained is ground, pelleted and stocked for marketing. It contains 16-20 % protein, 25-30 % cellulose and 100-150 mg carotene/kg. It is excellent fodder for cattle and rabbits.

For the profitability of this industry, the realization of the value of the two products (protein extract and co-product representing respectively 8 % et 92 % original DM) is essential.

1.6. **Homogeneity of Leaf Concentrate**

The protein content of LC varies slightly, from 50 – 60 % although the protein content of Lucerne as cut varies from 15 – 25 % according to the cut and vegetative stage of the crop. Thus the variation in quality of the vegetation processed results in a quantity of LC between 6 and 12 % of original DM with an average of 8 %.

1.7. **Village production** (schematic diag.)
Alongside this industrial production there is a village process, or « cottage industry », using a simplified system applicable in developing countries. In use in several « third world countries », this process is similarly based on heat coagulation.

For instance, in the method applied in Nicaragua by the association SOYNICA :

* Lucerne is washed, straight after harvest, in clean water,
* It is then pulped (vertical or horizontal pulper) and pressed in cheap and simple machines that can be made in good local workshops,
* The green juice containing the proteins is brought quickly to the boil,
* The floating protein curd is creamed off and then pressed to dewater it,
* The moist cake obtained can then be eaten directly or be dried, crumbled and kept in sealed packets. The co-product fibre can be used fresh by animals or sun-dried for later use. The brown juice can either be drunk by animals or used as fertiliser.

Comparatively, the quantity of protein recovered in this way is somewhat lower than that achieved industrially (about 5 %). The pressing is much less effective. Also the industrial LC is drier. However, the « village process » has the advantage of being able to be very simply set up in villages having available sufficient cultivable land and water.

III – USE OF LC AS ANIMAL FEED

The process of extraction of protein from lucerne was set up as an industrial pilot plant by the American researchers, Kohler and Bickoff at USDA (Albany, California). In 1972, FRANCE LUZERNE acquired the patents and was able to start industrial exploitation by 1975. This LC, called PX 1 has been used in France since then and is given to particular types of animal. It is nutritionally effective for monogastric animals such as the pig, due to the low content of indigestible fibre and richness in protein, vitamins and pigments. It is also very well made use of by poultry for its pigmenting ability (for the coloration of chicken flesh and egg yolks).

In the context of research into feed supplements, LC have been the subject of many studies at the Pig Breeding Research Station of INRA at Jouy en Josas, the pigs getting 6-8 g LC/day/kg live, enjoying increased growth rates. It seems also that LC, thanks to its low fibre content, shows very good protein digestibility (84 %) and that consumption of lucerne LC lowers faecal pollution for, for equal amounts of protein absorbed, the animal ingests rather less N.

Bourdon et al. (1980) also showed that in a wheat based diet PX 1 was an attractive protein supplement for pig growth. Calculation of its energy value shows that PX 1, at 10 – 20 % of the ration, has digestible energy of 3 735 kcal/kg DM (cf. 4 000 for soya) and metabolisable energy of 3 322 kcal/kg DM (cf. 3 750 for soya). The apparent utilisation coefficient of digestible energy (72,7 %) is very encouraging even if slightly inferior (by 2 %) to that of soya oil-cake. Thus, in association with wheat, lucerne LC is an ideal protein supplement for the growth of pigs in partial or total replacement of soya cake. The same applies to the combination maize-PX 1. Unfortunately, in present economic conditions PX 1 is not competitive for pigs.

Finally, throughout all those studies (Bourdon et al. 1980, Inra de Jouy en Josas) no sign of photosensitisation and no other side-effect (locomotor, pneumonia, diarrhoea or rectal prolapse) has been observed contrary to those noted by researchers in New Zealand in 1974 (Carr and Pearson) and the US in 1975 (Myers and Cheeke).

In 1975, FRANCE LUZERNE had been contacted by English researchers of the association « Find Your Feet » who were testing in India the supplementation of diets with LC from local vegetation and who wanted to test lucerne LC. The important richness of LC in protein, essential amino-acids, polyunsaturated fatty acids, vitamins and trace elements, and the good results obtained in animal feeding trials, led them to envisage its use as a nutritional supplement by populations often malnourished in developing countries. To help understand more fully its potential as human food, the nutritional composition of lucerne LC is developed in the following chapter.
**IV – COMPOSITION OF LUCERNE LEAF CONCENTRATE**

<table>
<thead>
<tr>
<th>Components</th>
<th>Content (g per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8</td>
</tr>
<tr>
<td>Crude protein (N x 6,25)</td>
<td>50 – 60</td>
</tr>
<tr>
<td>Fats</td>
<td>9 – 10</td>
</tr>
<tr>
<td>Glucides</td>
<td>6</td>
</tr>
<tr>
<td>Fibre</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Minerals</td>
<td>13 – 14</td>
</tr>
<tr>
<td>Vitamins</td>
<td>0.09</td>
</tr>
</tbody>
</table>

1. **Nutritional composition**

Lucerne LC is an interesting food from the nutritional view-point by virtue of its high content of protein, vitamins and trace elements (Table 1).

**Table 1 : Components of lucerne LC (Internal document FRANCE LUZERNE)**

Quantitative and qualitative analyses of the LC are regularly done on the final products, in the labs of FRANCE LUZERNE, under the direction of Mr SEILER, before they are put into storage, sampling each lot of 25 T. thus 30 000 samples are taken every year and about 130 000 analyses made.

1.1. **Composition in proteins and amino acids**

The proteins taken in as food are indispensable to ensure the renewal of the amino-acids required for the synthesis of the structural and functional cells of the body. Lucerne LC has 50 – 60 % crude protein (Total N x 6,25) of which 80 % is true protein, the remainder being free amino-acids, peptides, nitrogenous bases and traces of nitrates.

The most abundant of the proteins is a soluble chloroplastic protein of 500 kDa: RUBISCO (Ribulose – 1,5 biphosphate carboxylase-oxygenase). Numerous other soluble proteins with enzymatic functions are also there but in smaller proportion. Then, there are also some membrane proteins (molecular mass between 20 – 110 kDa) and some polypeptides from the hydrolysis of heavy proteins.

The amino-acids composition of LC is shown in Table 2 (see following page : internal document of FRANCE LUZERNE).

There are 3 categories of amino-acids :

- The non essential amino-acids (NEAA) which are synthesised directly by the body,
- The semi essential amino-acids (SEAA) synthesised only if their precursor is adequately present in the diet. This applies to cysteine and tyrosine.
- The essential amino-acids (EAA). there are eight of these (Table 2) which cannot be synthesised from other amino-acids or nutriments. It is imperative that they are sufficiently present in the diet or serious deficiencies result (retarded growth, loss of muscle, anaemia, lower resistance to infections...).
<table>
<thead>
<tr>
<th>Amino-acids</th>
<th>Eggs (g/%) #</th>
<th>Wheat (g/%) ##</th>
<th>Lucerne LC (g/%)</th>
<th>mg per 10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>3.4</td>
<td>4.1</td>
<td>4.8 - 6.3</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>5.6</td>
<td>3.4</td>
<td>5.9 - 7.1</td>
<td></td>
</tr>
<tr>
<td>Valine*</td>
<td>5.6</td>
<td>4.4</td>
<td>5.8 - 6.7</td>
<td>308</td>
</tr>
<tr>
<td>Leucine*</td>
<td>8.1</td>
<td>6.9</td>
<td>8.5 - 10.6</td>
<td>443</td>
</tr>
<tr>
<td>Isoleucine*</td>
<td>4.8</td>
<td>3.5</td>
<td>4.3 - 6.7</td>
<td></td>
</tr>
<tr>
<td>Methionine*</td>
<td>3.4</td>
<td>1.6</td>
<td>1.5 - 2.6</td>
<td>112</td>
</tr>
<tr>
<td>Cysteine**</td>
<td>2.7</td>
<td>2.4</td>
<td>0.6 - 3.0</td>
<td>59</td>
</tr>
<tr>
<td>Phenylalanine*</td>
<td>6.5</td>
<td>5.0</td>
<td>5.8 - 7.0</td>
<td>250</td>
</tr>
<tr>
<td>Tryptophan*</td>
<td>1.7</td>
<td>1.2</td>
<td>1.6 - 3.4</td>
<td>100</td>
</tr>
<tr>
<td>Proline</td>
<td>3.5</td>
<td>10.0</td>
<td>4.4 - 5.7</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>7.3</td>
<td>5.2</td>
<td>4.1 - 5.6</td>
<td></td>
</tr>
<tr>
<td>Threonine*</td>
<td>5.1</td>
<td>3.0</td>
<td>4.6 - 5.8</td>
<td>239</td>
</tr>
<tr>
<td>Tyrosine**</td>
<td>4.1</td>
<td>3.2</td>
<td>3.7 - 5.2</td>
<td>242</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.5</td>
<td>5.0</td>
<td>9.3 - 10.7</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.4</td>
<td>31.0</td>
<td>10.6 - 12.5</td>
<td></td>
</tr>
<tr>
<td>Lysine*</td>
<td>6.7</td>
<td>2.7</td>
<td>5.6 - 7.4</td>
<td>321</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.1</td>
<td>5.1</td>
<td>4.4 - 4.6</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>2.5</td>
<td>2.3</td>
<td>1.8 - 2.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Percentage of amino acid content in Lucerne LC (FRANCE LUZERNE)

*EAA, **SEAA, # INRA data, ## INAPG data.

Figures in g for 100 g proteins (eggs and wheat) and for 100 g amino acids (LC)

It may be worth remarking that a study led by the Nutrition Lab. of NANCY (Debry, 1977) found tryptophan levels of 3.42.

Overall, the EAA and SEAA represent 50% of the total amino-acids of LC. They would therefore make a useful contribution to human nutrition.

However, few measures of digestibility of the amino-acids and proteins of leaves have been undertaken, and we know that certain constituents, such as polyphenols, tannins and fibre do limit the digestibility and assimilation of vegetable proteins.

Nonetheless, the biological value of the legumes, characterised by their tendency to be more or less well assimilated and to promote the synthesis of other amino-acids, lies between 70 and 85%. Then again, two foods, each deficient in an EAA, may complement each other, if the limiting factors are different. This type of combination can not only remove deficits but also can increase the biological value of the relevant proteins, providing a contribution sometimes superior to the best animal proteins. For example, the combination of legumes with whole cereals (limited by lysine and by tryptophan in maize) lifts the biological value of the proteins from 30 to 50%.

That is why the combination of cereals and leguminous grains is at the root of many dietary traditions (France: bread + soup with haricots, lentils or broad beans; Italy: pasta + haricots; Maghreb: couscous + chick peas; Japan: Rice + soya sauce...). For good health it is important to diversify the vegetables to ensure a balanced protein intake. In fact, the protein ration best adapted to human metabolism (taking account of the ideas of...
EAA, of limiting factors and of losses) consists of 2/3 legumes, whole cereals, oily fruit, eggs and dairy products, with a smaller proportion (1/3) of fish and meats (Le Goff, 1997).

1.2 Lipids
Lucerne LC contains on average 8 – 12 % lipids as fatty acids, glycerides, pigments, sterols and fat soluble quinones, coming mainly from the chloroplasts. The lipids are very important for the body as they are involved in the making of hormones, prostaglandins and are also necessary for the absorption of some fat soluble vitamins (A, D, E and K) ingested (Douillard, 1981; Le Goff, 1997).

1.2.1 Fatty acids
Within the body, several types of fatty acid are found:

* Saturated fatty acids, which play a basic role in the membrane structure of the cells of the central nervous system. Their intake is imperative in children and pregnant women. In lucerne LC, the principal ones are palmitic and stearic acids, respectively 25 % and 5 % of the total fatty acids.

* Unsaturated fatty acids, which exist in two forms:
  
  ▷ Mono-unsaturated, such as oleic acid, which has a very beneficial influence on the factors of coagulation and in the prevention of cardiovascular ailments because of its atherogenic action. In LC it forms 8 % of total lipids.

  ▷ Poly-unsaturated, which are essential for the metabolic reactions in which they are involved. It is imperative that some poly-unsaturated fatty acids, such as linoleic an linolenic are in the diet for the body is unable to synthesise them. These two compounds are the precursors of two important metabolic sequences (respectively Omega 6 and Omega 3). Human physiology relies on the balance between these two EAA.

However, it must be remembered that an excess of fatty acids may sometimes be harmful. These compounds are in fact easily oxidized by metallic cations or metalloproteins. Such oxidation gives rise to free radicals and peroxides which can be toxic to cell membranes.

Apart from their effect on the cardio-vascular system and on inflammation reactions, all these fatty acids act upon the permeability of cell membranes, the transmission of nerve impulses, the metabolism of adrenal hormones and male fertility (Le Goff, 1997). They are therefore of great importance physiologically, both to animals and man.

1.2.2. Pigments
The principal pigments found are the chlorophylls (c.1 %) giving the LC its green colour, and the carotenoids (2 – 3 g/kg). Amongst these latter the main ones are B-carotene or pro-vitamin A (0.5 g/kg) and xanthophylls: lutein (600 mg), zeaxanthin (200 mg) violaxanthin and neoxanthin (200 mg). Those molecules may be either free or associated with proteins.

The stability of the carotenoids in LC is of much more importance than in the dried whole crop, for the artificial drying of lucerne largely stabilises the pigments (Abely, 1995).

1.2.3. Sterols
Sterols are less abundant. They are principally cholesterol and betasitosterol. The latter, present in the chloroplasts, is hypocholesterolemic: it diminishes intestinal absorption of cholesterol and so lowers the blood cholesterol level.

1.2.3. Quinones
The most abundant quinones are ubiquinone, alpha-tocopherol (precursor of vitamin E) and phylloquinone (vitamin K). However they amount to no more than 0.03 – 0.05 % of the nutrients in LC (tab. 3).
1.3. Carbohydrates

Sugars are the body's principle and most economical energy source. The consumption of energy in the form of glucose is more or less constant and requires a regular intake of glucides in food. Only the simple sugars (-oses) are directly assimilable by the organism. Complex sugars (-osides and –heterosides) are progressively broken down with the aid of catalysts in the original vegetation (vitamins, trace elements, enzymes). Thus made available to the body, they are absorbed over time to ensure a regular energy supply provided that they are accompanied by dietary fibre.

A balanced diet will have c.60 % of its energy supply as glucides, mainly as complex sugars (particularly the starch of cereal and leguminous grains) and c.40 % as simple sugars (Le Goff, 1997). In LC, the glucides appear in two forms:

- Simple sugars: glucose (0.8 %)
- Complex sugars: saccharose (0.3 %); stachyose (0.1 %), glucosanes (3.2 %), pentosanes (2 %), galactanes (2.7 %) and mannanes (0.1 %).

In all, these sugars account for 6 % of the content of LC (internal documentation FRANCE LUZERNE). Taken as a supplement in the recommended dosage (6-15 g LC/day) this can help stabilize glycaemia.

1.4. Fibres

Lucerne LC has less than 2 % of fibre as cellulose, hemicellulose, polymerised sugars and lignin (internal documentation FRANCE LUZERNE). This low fibre level both allows the concentration of useful components (vitamins, minerals…) and improves their assimilation in the digestive tract. The fibre necessary for the good functioning of the bowel is adequately supplied by the more or less whole cereals normally eaten.

1.5. Vitamins

Vitamins, like minerals, affect both the good assimilation and utilisation of foodstuffs. Their catalytic activity makes them intervene in the assortment of biochemical reactions of the body. They are divided into two groups by solubility: those that are lipid soluble (and so storable in body fat) A, D, E and K; and those that are water soluble B, C and P, which are easily eliminated and so must be taken regularly in the diet.

Lucerne is a good source of B-carotene (precursor of vitamin A) and also of other vitamins such as E, K and B9. Each of these has very important specific functions in the body.

There is also in lucerne LC some choline chlorhydrate at the level of 6.4 mg/10 g LC (0.064 %). The importance of this component merits deeper study.

<table>
<thead>
<tr>
<th>Component</th>
<th>Function (*)</th>
<th>Daily Requirement (*)</th>
<th>Content in 10 g Lucerne LC (**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-carotene</td>
<td>Synthesis of retinal pigment</td>
<td>Child, 1-3 yrs: 400 µg RE</td>
<td>920 µg RE</td>
</tr>
<tr>
<td>(pro-vitamin A)</td>
<td>Cellular activity (division, permeability, tone)</td>
<td>Child, 4-9 yrs: 600 µg RE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antioxidant</td>
<td>Adolescents: 800 µg RE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-infective</td>
<td>Adults: 800-1300 µg RE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-atheromotic</td>
<td>RE = Retinol Equivalent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assists detoxification of the liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Antioxidant</td>
<td>Child, 1-3 yrs: 5 mg</td>
<td>3 mg</td>
</tr>
<tr>
<td></td>
<td>Fertility</td>
<td>Child, 4-9 yrs: 7 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diminishes oxygen demand</td>
<td>Adolescents: 10 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nutrition of conjunctive tissue, muscle and skin</td>
<td>Adults: 12 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resists destruction of red corpuscles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Vitamin K
- Anti-haemorragic (activates synthesis by the liver of coagulants)
  - Child, 1-3 yrs: 15 µg
  - Child, 4-9 yrs: 25 µg
  - Adolescents: 35 µg
  - Adults: 35-55 µg

Vitamin B9 (folic acid)
- Complement of vitamin B12
- Synthesis of haemoglobin
  - Child, 1-3 yrs: 100 µg
  - Child, 4-9 yrs: 200 µg
  - Adolescents: 300 µg
  - Adults: 300-500 µg

| Table 3: Content and characteristics of the principal vitamins present in Lucerne LC |
| (*) Le Goff, 1997; (**) APEF data |

1.6. Minerals

Mineral matter in LC averages 13 – 14 % of DM. It is largely water soluble and may be partly removed by acidwashing at pH 3-4 (Analyses by INRA; internal documentation FRANCE LUZERNE).

The minerals are listed in Table 4. They are active in many reactions and in tissue building. The role of each element is not yet very well defined: some are merely catalysts, others have both plastic (tissue structure) and catalytic functions. It must be said, too, that some plant acids form with minerals insoluble salts which makes them unavailable (e.g. oxalic acid in some leaves and phytic acid in cereals).

All these elements have important functions. They are none of them synthesised by the body and so must be taken in food.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Function</th>
<th>Daily Requirement</th>
<th>Content in 10 g Lucerne LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>Formation and growth of bones &amp; teeth, Catalyst (blood coagulation)</td>
<td>Child, 1-9 yrs: 600-700 mg, Adult: 900 mg, Adolescent, elderly, lactating women: 1 200 mg</td>
<td>320 mg</td>
</tr>
<tr>
<td>Phosphorus (P) (non-phytic)</td>
<td>Formation and growth of bones &amp; teeth, cell membranes, Fat metabolism, Catalyst (phosphorylation)</td>
<td>Child, 1-9 yrs: 500-600 mg, Adult: 800 mg, Adolescent, elderly, lactating women: 1 000 mg</td>
<td>70 mg</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>Principal intracellular cation, Assists in regulating acidic/basic balance, Active in transmission of nerve impulses</td>
<td>1 000 – 2 000 mg</td>
<td>70 mg</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>Principal extracellular cation, Antagonistic &amp; complementary to K</td>
<td>3 000 – 5 000 mg</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>Formation &amp; growth of bones &amp; muscles, Activates ATP in the energy cycle, Activates numerous enzymes</td>
<td>Child, 1-9 yrs: 120-180 mg, Adult: 340-420 mg, Adolescent, elderly, lactating women: 330-480 mg</td>
<td>13 mg</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>Haemoglobin synthesis, Essential ion for respiration, Anti-infective</td>
<td>Child, 1-9 yrs: 10 mg, Adult: 10-18 mg, Adolescent, elderly, lactating women: 10-18 mg</td>
<td>7 mg</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>Formation and growth of skin, bones, nails and hair, Variously catalytic</td>
<td>Child, 1-9 yrs: 10 mg, Adolescent, Adult: 12-19 mg</td>
<td>200 µg</td>
</tr>
<tr>
<td>Mineral</td>
<td>Function and Content</td>
<td>Recommendations</td>
<td>RDA</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Mangenese (Mn)</td>
<td>Promotes redox reactions&lt;br&gt;Antioxidant, anti-allergic, anti-infective&lt;br&gt;Bone mineralization</td>
<td>Child, 1-9 yrs: 1-2 mg&lt;br&gt;Adolescent, adult, elderly, lactating women: 4 mg</td>
<td>600 µg</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>Cell respiration&lt;br&gt;Synthesis of haemoglobin and vitamin C&lt;br&gt;Anti-infective, anti-inflammatory</td>
<td>Child, 1-9 yrs: 1-1.5 mg&lt;br&gt;Adolescent, adult, elderly, lactating women: 2-3 mg</td>
<td>78 µg</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>Regulates Synthesis of vitamin B12&lt;br&gt;Antispasmodic &amp; sedative to the autonomous nervous system</td>
<td></td>
<td>2 µg&lt;br&gt;10 µg</td>
</tr>
<tr>
<td>Iodine (I)</td>
<td>Thyroid hormones (activation of all metabolism)</td>
<td>Child, 1-9 yrs: 70-120 µg&lt;br&gt;Others: 150-200 µg</td>
<td>3 µg</td>
</tr>
<tr>
<td>Selenium (Se)</td>
<td>Anti-oxidant&lt;br&gt;Anti-inflammatory&lt;br&gt;Stimulates the immune system</td>
<td>Child, 1-9 yrs: 20-30 µg&lt;br&gt;Others: 55-75 µg</td>
<td>0.5 – 1 µg</td>
</tr>
</tbody>
</table>

Table 4: Function and content of Minerals in Lucerne LC (internal document FRANCE LUZERNE)

1.7. Energy value

Gross calorific value: 4 700 kcal/kg.<br>Metabolisable energy (poultry): 2 600 kcal/kg<br>Metabolisable energy (pigs): 3 700 kcal/kg.

1.8. Study of variability of results

A study requested by APEF looked at the change over the years in the composition of dried lucerne LC (Table 5).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture g/kg</td>
<td>92.1</td>
<td>81.4</td>
<td>88.2</td>
<td>89.1</td>
<td>85.9</td>
</tr>
<tr>
<td>Protein N x 6.25</td>
<td>526</td>
<td>553</td>
<td>550.1</td>
<td>554.5</td>
<td>563.5</td>
</tr>
<tr>
<td>Fibre g/kg</td>
<td>23.5</td>
<td>16.8</td>
<td>17</td>
<td>13.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Lipids g/kg</td>
<td>74.6</td>
<td>108.6</td>
<td>129.8</td>
<td>90.3</td>
<td>108.2</td>
</tr>
<tr>
<td>Crude Ash g/kg</td>
<td>106.3</td>
<td>96.9</td>
<td>80.9</td>
<td>112.1</td>
<td>106.4</td>
</tr>
<tr>
<td>Beta-carotene mg/kg</td>
<td>100.36</td>
<td>71.55</td>
<td>103.97</td>
<td>145.87</td>
<td>603.77</td>
</tr>
<tr>
<td>Available xanthophylls mg/kg</td>
<td>704.74</td>
<td>631.84</td>
<td>743.68</td>
<td>781.53</td>
<td>1727.5</td>
</tr>
<tr>
<td>Phosphorous g/kg</td>
<td>8.43</td>
<td>6.93</td>
<td>7.34</td>
<td>8.09</td>
<td>8.32</td>
</tr>
<tr>
<td>Calcium g/kg</td>
<td>33.92</td>
<td>31.53</td>
<td>25.7</td>
<td>35.78</td>
<td>33.8</td>
</tr>
<tr>
<td>Magnesium g/kg</td>
<td>1.52</td>
<td>1.25</td>
<td>1.47</td>
<td>1.39</td>
<td>1.45</td>
</tr>
<tr>
<td>Potassium g/kg</td>
<td>9.81</td>
<td>6.47</td>
<td>9.06</td>
<td>7.29</td>
<td>6.6</td>
</tr>
<tr>
<td>Sodium g/kg</td>
<td>0.06</td>
<td>0.03</td>
<td>0.07</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Iron mg/kg</td>
<td>507.02</td>
<td>595.75</td>
<td>435.38</td>
<td>754.79</td>
<td>623.4</td>
</tr>
<tr>
<td>Manganese mg/kg</td>
<td>57.18</td>
<td>64.89</td>
<td>54.17</td>
<td>65.57</td>
<td>67.1</td>
</tr>
<tr>
<td>Copper mg/kg</td>
<td>6.67</td>
<td>8.1</td>
<td>7.88</td>
<td>7.48</td>
<td>7.8</td>
</tr>
<tr>
<td>Zinc mg/kg</td>
<td>17.15</td>
<td>16.21</td>
<td>36.44</td>
<td>16.85</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Table 5: Comparative study of the composition of samples of lucerne leaf concentrate produced from 1992-1996 (analysis in 1997 after storage in open sacks)
The samples had been taken from unsealed bags opened in the year shown. Each extract came from cuts made in August, September or October 1992-1996 (internal documentation FRANCE LUZERNE).

It is clear that the % of protein, xanthophyll and B-carotene increased from year to year while the cellulose level fell progressively. This was due to optimisation in the whole production sequence. Other components were relatively uniform through the period, except for the carotenoids. In these, a natural variation over time is seen, due to atmospheric oxidation. For this reason, an antioxidant (usually ascorbic acid) is added during production. Remember that the results in table 5 are for open bags and that oxidation is less with sealed bags.

2. **Toxins and anti-nutritional factors**

The analysis of antinutritional factors is the subject of a FRANCE LUZERNE research programme with advice from the Centre for Analysis and Industrial Evaluation of Agricultural substrates (CAVISA of REIMS - Mme Hennequin).

2.1. **Analysis of heavy metals and pesticide residues**

None of the analytical results on FRANCE LUZERNE's LC has exceeded the permissible levels for toxic components such as pesticide residues, heavy metals. Of course, the making of LC is done from lucerne having had at most one chemical treatment (a weed killer and, rarely, an insecticide) before the start of regrowth. The spreading of sludge and urban compost (often vectors of heavy metals) is moreover forbidden. The only antinutritional components likely to be present are naturally produced in the plant: saponins, polyphenols and proteinaceous anti-tryptic factors in unheated products. But, in the process, the LC is heated to above 90°C.

2.2. **Analysis of naturally occurring antinutritional factors**

Some naturally occurring compounds in the plant can have negative effects if consumed in quantity. However the rations of LC given to animals (6-8 g/day/kg live wt, Bourdon et al. 1980) and the intakes recommended for people (0.6 g/day/kg body wt for children and 0.15 – 0.3 g/day/kg for adults) are very low.

2.2.1. **Saponins**

Some saponins sometimes slow down the growth of young animals by forming a complex with cholesterol which inhibits its assimilation. This has been seen in rats and poultry but not in pigs (FRANCE LUZERNE; INRA).

In lucerne LC, the level of saponins (steroid and triterpene) found does not exceed 1 % (0.7 – 0.9 g per 100 g product) and varies only slightly with the genetic origin of the lucernes.

2.2.2. **Polyphenols**

Polyphenols are chemically highly active compounds that can be at the root of several undesirable effects:

- Diminution of the absorption of Fe,
- Oestrogenic effect of coumesterol (0.14 – 0.43 %) or of isoflavones (0.7 – 2 %)
- Diminution of protein digestibility. Polyphenols are well known to react with and form strong covalent bonds with the ε-amino groups of lysine.

In fact, only traces of chlorogenic acid strongly bound to proteins of low molecular wt. have been found (Abely, 1995).

2.3. **Bacteriological analysis**

In 1993, a bacteriological check was done at the European Institute for the Environment at Bordeaux (IEEB, Health and Hygiene lab.) on samples of another series of analyses of PX granules. Another series of analysis of 3 samples has recently been done there and at the Institute of Hygiene and Public Health Research, Nancy. The results are shown in Table 6.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of storage</td>
<td></td>
<td>1 month</td>
<td>3 months</td>
<td>1 year</td>
</tr>
<tr>
<td>AFLATOXINS (mg/kg) *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins B1</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins G1</td>
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<td></td>
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<tr>
<td>Aflatoxins G2</td>
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<td></td>
<td></td>
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<tr>
<td>BACTERIOLOGY **</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Aerobic mesophilic flora (CFU/g)</td>
<td>3050</td>
<td>7950</td>
<td>6000</td>
<td></td>
</tr>
<tr>
<td>Total coliforms (CFU/g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fecal coliforms (CFU/g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Salmonella spp. (CFU/g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Staphylococci (presumed pathogenic) (CFU/g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Colstridium spores sulphite-reducing (CFU/g)</td>
<td>20</td>
<td>35</td>
<td>75</td>
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<tr>
<td>MYCOLOGY * (count/g)</td>
<td></td>
<td></td>
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<tr>
<td>Moulds &amp; yeasts on agar</td>
<td>3700</td>
<td></td>
<td></td>
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<tr>
<td>Aspergillus gr. glaucus</td>
<td>3000</td>
<td></td>
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<tr>
<td>Aspergillus versicolor</td>
<td>3000</td>
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<tr>
<td>Aspergillus candidus</td>
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<tr>
<td>Scopulariopsis brevicaulis</td>
<td>500</td>
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<tr>
<td>Penicillium cyclopium</td>
<td>50</td>
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<tr>
<td>Absidia corymbifera</td>
<td>50</td>
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<tr>
<td>Cladosporium cladosporioides</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>METALS **</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni threshold : 3.9 µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd threshold : 3.1 µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb threshold : 2.2 µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As threshold : 1 µg /kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg threshold : 3.9 µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organochlorines-Organophosphates</td>
<td>Not detectable</td>
<td>Not detectable</td>
<td>Not detectable</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Results of bacteriological analyses, 18/02/93, of coarsly ground PX and of samples from the seasons of 1996/1997 and 1997/1998. The threshold is the maximum permitted level duly authorized.

2.4. LC from other plants
Many scientists have remarked that a lot of plants (Egyptian clover, niébé bean, mung bean, sesame, amaranthus) have characteristics similar to those of lucerne, particularly in their content of protein, amino-acids, vitamin and iron.

Thus for many years the English Charity « Find Your Feet » (known in France and the USA as « Leaf For Life ») has used the high potential of LC from local vegetation (and sometimes from lucerne) to tackle malnutrition. Using a simple and cheap « village process » they have recorded an improvement in the health of children and of adults suffering the ailments allied to malnutrition (apathy, anaemia, xerophthalmia, respiratory, digestive and nervous troubles).

Because of its good nutritive and hygiene quality, lucerne LC (taken as a food supplement, especially with cereals) should be of considerable interest for human health in view of the tables above and the summary of results below.

V – SIGNIFICANCE OF LC IN HUMAN NUTRITION

FAO has, for long, tried to define criteria for quantifying malnutrition.

In the 70’s it put the accent on protein deficit. Later, it defined malnutrition (or chronic under-feeding) by the single deficit of energy when the level is below an annual average intake of 2 200 cal/day. 780 million individuals are concerned, of whom 195 million are children under 5 (FAO/WHO, 1992). However, this method does have the disadvantage of failing to take into account deficiencies in protein, vitamins and minerals. The main ones being iron, vitamin A, iodine and calcium. Now, some populations with low income, while satisfying the criterion of 2 200 cal, suffer shortages of nutrients. This refers to a much greater number than that estimated by FAO: more than 2 000 million individuals are affected by these two forms of malnutrition: caloric and specifically deficient in one or more nutrients.

Specific nutritional deficiencies can cause serious health problems, especially in children: low weight, slow growth, mental retardation, weakened immunity, sometimes death. In fact, it is during the first 5 years that the consequences are most dramatic: the body and especially the nervous system are in a period of rapid growth and need the presence for their development of a proper assembly of nutrients.

Relevance of LC:

The use of LC can be a response to many of these specific deficits. Since 1960, the English researchers, Waterlow and Pirie, have been spreading this idea by using, in developing countries, various LC got from local green leafy vegetables. In fact, after the discovery by Rouelle (1773) of vegetable protein it had to wait for the work of Osborne and Wakeman (1920) to be taken up by Pirie in 1940 for a process of precipitation and separation of LC to be perfected. And it was Guha in 1943 who, for the first time, in a famine in Bengal, applied it to human feeding using leaves of cereals and water hyacinth.

1. Associations fighting malnutrition

The struggle against malnutrition is an almost insurmountable economic and socio-political problem: the need is huge, the costs very high and the available funds inadequate. A solution can only be found in food self sufficiency. Two associations, one English (Find Your Feet), the other French (APEF) champion the idea of production self-sufficiency through using LC from the vast range of plants available on earth, with the objective of tackling the deficits of protein, vitamins and minerals.

1.1. Find your Feet (Leaf For Life)

In 1967, the English Charity Find Your Feet (previously working on the rehabilitation of displaced persons) decided to support Professor Pirie and Waterlow in their research on LC, and launched a programme under the title (Leaf for Life), which name was adopted by their sister organization in the USA.

Since then, FYF has collaborated in and financed trials by English and Indian researchers which have developed the « village process » using very simple technical procedures adapted to regional climates and customs. They have set up such units in many countries, such as Mexico, Sri Lanka, India, Bangladesh, Bolivia and Nicaragua.
1.2. APEF

It is with the same perspective, but with the industrial approach in mind, that APEF was created in 1993, on the initiative of Mr Jacques Subtil (President), Mme Irène Gastineau, M. Roger Douillard, M. Olivier de Mathan and M. Jean-Marie Mojon.

With the help of numerous medical and agricultural personalities (Annex 1) APEF is conducting studies and trials of the supplementation of deficient diets in different countries using lucerne LC made in France. The three main lines at the moment are:

[*] In Romania (trials since July 1994, supported by the Order of Malta and Professor Atanasiu, microbiologist and WHO consultant),

[*] In Nicaragua where the Association SOYNICA, since 1995, has supplemented the diet of 3 000 children under 6 and of pregnant women and lactating mothers,

[*] In China in cooperation with researchers at the Universities of Beijing and Perugia since 1996.

The Association, consisting of voluntary workers, gets support from FRANCE LUZERNE and its 3 production plants who supply APEF with LC for the trials. It is also supported by the Order of Malta and Rotary, but has so far not received recognition from official bodies such as FAO and WHO.

2. Methodology and results

The dose recommended by Professor Jean-Claude DILLON, nutritionist, for lucerne LC as a dietary supplement is 10 g/day for children of 10 kg and more. For adults, pregnant women and the aged, a daily dose of 10 – 15 g seems good. In Nicaragua, on weaning at 6 months, infants are given LC mixed with liquid honey.

In a few people, starting to take lucerne LC can give digestive troubles, so doses are increased successively: 2.5 g/day initially, with 2.5 g increments. LC is generally mixed with local dishes (soups, biscuits, pasta, cakes, drinks, maize or wheat flour, cream cheese...) to avoid damage to vitamins, it is best added after cooking.

Theoretically, 10 g LC (Table 7) will supply:

[*] 300 % of child’s requirement of vitamin A (as it is in the form of B-carotene, there is no risk of an excess of vitamin A),

[*] 100 % of his iron,

[*] 50 % of his folic acid (vitamin B9),

[*] 40 % of his vitamin E,

[*] 20 % of his protein.

<table>
<thead>
<tr>
<th>Component (per 10 g)</th>
<th>Nutrients provided</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protéins (4.9 – 5.3 g)</td>
<td>Lysine</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td>Tryptophan</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>Cystein</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
<td>250</td>
</tr>
<tr>
<td>Lipids (1 g) among which are</td>
<td>Linolenic acid</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid</td>
<td>133</td>
</tr>
<tr>
<td>Minerals (0.9 – 1.3 g)</td>
<td>P (available)</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table 7: Nutritional content of 10 g lucerne leaf concentrate – APEF data

<table>
<thead>
<tr>
<th></th>
<th>Mg</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>0.078</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Beta-carotene</td>
<td>920 µg RE</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Vitamin B9</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Vitamin K</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Choline chloride</td>
<td>6.4</td>
</tr>
</tbody>
</table>

2.1. Effect on anaemia

The most widespread result of malnutrition in the world is anaemia. In the 3rd world, it afflicts about 500 million children and 60% of pregnant women (FAO/WHO 1988). Altogether 2 000 million people (mainly women and pre-school children) are concerned (FAO/WHO 1992).

According to WHO, anaemia is characterized by Hb levels less than 11 g/dl of blood in young children, 12 g/dl in older children and adults (WHO 1972). Anaemic children are both smaller and more susceptible to infection (through immunodeficiency) than are others. Growth is retarded both by the malnutrition itself and by the many resulting illnesses (Chwang and al. 1988). Such children are also less attentive at school and show mental development inferior to other children's (Pollitt and al. 1986).

In most cases, the low Hb levels found are the result of diets low in iron and protein, often currently eaten in developing countries. However, deficiencies of folic acid, vitamins A, B2, B6 or B12 (in the case of pernicious anaemia) and of copper may be responsible as well (Passmore and Eastwood, 1986). The low standard of living of these populations does not allow them to buy the animal products or fruits and vegetables that could make up for the limited range of amino-acids, vitamins and trace elements in grains. But, as we have seen, 10 g lucerne LC has on average 7 mg Fe and many other nutrients.

* FYF has shown that, in Bolivia, Nigeria and Pakistan:
  - a dietary supplement of milk or LC in young children gives significant improvement in growth when compared with WHO standards. The weight gain was also constantly considered due to a lowered incidence of infections,
  - Daily distribution of LC or milk to infants with kwashiorkor was also accompanied by spontaneous remission of anaemia (Olatunbosun, 1972; Shah et al, 1981). Their Hb levels rose. The simultaneous taking of vitamin C increased this effect: the most probable explanation being that the Non-haem iron of the LC was better absorbed when reduced (Lowe, FYF, 1992). Ascorbic acid tends to keep the iron in this state (Chanarin, 1988).

* APEF, too, has conducted trials with LC produced by FRANCE LUZERNE:
  - The same positive effects were shown in China in students and others aged from 14 – 65 (GUO Pei yu, 1996); in people aged 50-86 in Romania (Zeana, 1996); in children under 2 by the Institute for the Protection of Mother and child, Bucarest (Georgescu and Nanu, 1997); in 200 children from 6 months to 6 years of age and in pregnant women and lactating mothers given, for 4 months, a daily supplement of 10 g lucerne LC in Nicaragua and Romania (Teran Hidalgo 1996; Zeana, 1996).

However, the bioavailability of the iron in LC has not yet been studied. It seems, nevertheless, to be higher than that normally accepted for the absorption of vegetable iron.

It has been found in all the blood sampling done so far that adding LC to traditional diets has raised the Hb levels and has reduced or eliminated anaemia.
2.2. Effect on vitamin A deficiency

According to UNICEF (1988), vitamin A deficiency is responsible, annually, for the blindness of more than ½ million children of whom more than half die within a few months. It seems too, that lack of vitamin A influences the frequency of the diarrhoeal infections responsible each year for the death of 4 million children (Sommer and al. 1984, UNICEF). Now, in these countries, fruit and vegetables are expensive : people are unable to eat things rich in B-carotene or pro-vitamin A.

A study carried out over 3 months with young children showed both that their serum retinol level rose and that the absorption of B-carotene from LC (5,5 mg in 10 g LC DM) was 90 % (Devadas et Murthy, 1978). An identical result was found in old people in Bucarest (Zeana, 1996).

It further seems that regular supplementation with LC diminishes the degeneration of conjunctive tissue (Mathur, 1996) as well as the frequency, duration and intensity of diarrhoea (Study in Brazil, FYF, 1993).

2.3. LC and mental development

The development of the nervous system (in particular, the brain) of the child occurs from conception to the age of 5. At 2 years, brain weight is already 80 % of that in the adult. Malnutrition during this period can interrupt brain growth and cause serious and permanent lesions. Further continuing malnutrition can cause serious trouble even after the age of 2. However, if the diet is improved by the 3rd year growth can recover. But after the age of 5, previously inflicted restrictions of brain growth can no longer be remedied.

Studies on 2 000 people (young children and pregnant women) in Guatemala have shown how infant malnutrition handicaps the intellectual capacity of the future adult, his quality of life and productivity in work (Brown and Pollitt, 1996) :

- Infant mortality of children given a high protein supplement (Atole) fell by 69 % while their rate of growth increased compared with those on a high calory, non-protein, supplement.
- The children on Atole performed better in IQ tests than a control group on an energy rich supplement (Fresco) based on fruit and sugar.
- The protein supplement, Atole, improved learning ability : the longer they were at school, the greater was the difference in IQ between the two groups.

In Nicaragua, infants are traditionally weaned on to a maize porridge and SOYNICA supplements this with LC. On this topic, Professor Lestrade (pediatrician and nutritionist of the Academy of Medicine, now deceased) stressed : « the development of the nervous system is governed by the presence in the diet of iron and of amino-acids (especially tryptophan) in adequate amounts. The seeds, which are often the sole food, offer a poor supply of these. Infants, at weaning and in their early years, therefore risk suffering an irreversible mental handicap. LC is capable of correcting the nutritional deficiencies linked with seed eating thanks to its richness in tryptophan and iron ». 

2.4. Acceptability

The LC has the dark green colour of the lucerne leaves, it smells of the cut plant and has the characteristic flavour of spinach. These three factors can handicap its consumption among children even though the daily dose is low (6 – 10 g).

In Africa, traditional cooking favours acceptability since dishes are often flavoured with dried leaves. Elsewhere APEF leaves to local colleagues the devizing of suitable presentation ; thus :

- In Romania : it is mixed with rice porridge or cottage cheese and sugar,
- In Nicaragua : it is mixed with maize flour and sugar, or, for infants, with honey.
- In China : with soya flour.

All these feeding programmes require total dedication from the local officials, especially the doctors and health workers. The introduction of a new food product actually needs both very careful technical and psychological approaches to avoid set-backs.
2.5. **Side effects**

There have been but three reported cases of allergic reactions to LC; these were all of products coagulated at 75-80°C in the village production (Waterlow, 1962; Singh, 1971). Since then, no side effects have been observed in children or adults because the production process was modified: the juice is now heated to a minimum of 90°C and usually just brought to the boil. This both prevents formation of pheophorbide and kills pathogenic bacteria.

In the industrial process, there is no risk of inadequate heating. To date, almost 50 tonnes of LC (i.e. more than 5 million portions) have been eaten with no reported problem of intolerance or digestive disorder, as long as the intake has been graduated to start with. Just a few individuals have, in this initial phase, had minor digestive problems, always disappearing after a slightly prolonged accommodation period.

**VI – RESULTS AND DISCUSSION**

1. **Results**

The many studies carried on by FYF and APEF have by now shown that, in undernourished mothers, children and old people, LC, due to its high content of protein (especially Lysine and Tryptophan), vitamins and trace elements (especially iron and calcium) has several good effects:

- It encourages height and weight gain,
- It raises Hb levels and reduces or eliminates anaemia,
- It raises serum retinol levels and thus prevents the maladies associated with vitamin A deficiency,
- It encourages normal brain growth in children,
- It improves general clinical status and lowers both the frequency and severity of infections,
- No long-term side-effects have been found.

In developing countries, therefore, lucerne LC can be a very healthy nutritional supplement to cereal-based diets. By improving the physical and mental growth of children, it would raise the quality of life and the future productivity of such communities. The production process is very simple and cheap (about 30 FF or 5 USD/child/year). The available supply of nutrients in leaves is enormous (more than in seeds), renewable and inexhaustible.

**This use of Lucerne LC lets us entertain the hope that impoverished communities might themselves take it up to make LC from local greenstuff and make use of the by-product (fibrous residue and serum) for livestock.**

FYF has put together an annotated bibliography of the very encouraging results got with LC made by the village process, mainly used in supplementing childrens’ diets.

With the industrial LC, whose controlled quality provides both an assurance of production and longer term storage, **APEF has confirmed its nutritional efficacy in Nicaragua (children aged 6 months – 6 years and mothers), China (in adults) and Romania (in the aged).**

In developed countries, lucerne LC would help the fight against the malnutrition allied to family break-up, and against the changes in general condition (chronic fatigue, mental and physical overwork, spells of convalescence…) resulting from imbalances in the modern diet. All these conditions have in common excess of free radicals due to hyperoxidation and acidification in the gut. LC is rich in antioxidants (vitamin A and E, flavonoids, Selenium, Zinc and Copper).

**LC, as a nutritional supplement, can thus contribute to the betterment of the malnourished and the poor in reestablishing their dietary balance. It can likewise be valuable in industrialized countries by providing stimulating and stabilising factors introduced in various ways into diets.**
2. **Outlook and development strategy**

2.1. **Nutritional study of LC**

APEF envisaged two stages in the study of LC from the nutritional and dietary points of view:

1. **Confirmation of the efficacy of LC**
   - Observation of results,
   - Comparison of results obtained with dried LC with those got by FYF,
   - Scientific publication.

As we have already seen, this stage is now at an end since the 10 or so completed studies have all demonstrated the effectiveness of LC against anaemia (the blood level of Hb having been the sole criterion adopted for this stage).

2. **Scientific evaluation of the nutritional significance of leaf concentrate in nutrition**

The next phase will be the scientific evaluation of leaf concentrate. This will start soon by the study at CHU, Reims under the direction of Professors J.C. Etienne, S. Kochmann, M. Leutenegger, H. Choisy, P. Bouchet and Dr Kariger, of the bioavailability in healthy men of three important constituents: protein, iron and calcium.

Two projects, meanwhile, are being set up:

- in Mali, on the initiative of the Ministry of Health with the support of the Order of Malta, and
- in Mexico, with the Association Franco-Mexicana-Suiza y Belga de Beneficiencia de Mexico.

Their programme is in each case the same:

- The local organization is interested in leaf concentrate and wants to study its effect on a malnourished population with the aim of encouraging local production in the medium term.

- APEF offers to collaborate and to supply the required leaf concentrate free of charge (except for transport costs) as well as full information on the product and its use.

- The local organization is responsible for carrying out, with its own nutritionists and doctors, a supplementation trial of several months on a group of infants, children, mothers and/or aged.

- The criteria are simple: general clinical condition of each individual, haemoglobin level before and after the trial, record of illnesses throughout (type, duration and frequency), particularly for diarrhoea, respiratory troubles and night blindness.

It is to be expected that, at the end of the experimental period, those in charge will be in favour of a follow up programme for local production from vegetation adapted to the country's climatic and agricultural conditions.

2.2. **LC in developing countries**

The eradication of malnutrition, in the long term, cannot be achieved by importing food. It will come only by each region attaining self-sufficiency.

Likewise for successful development of the use of LC it is essential that eventually indigenous production takes place near places of need and centres of population. Two methods are seen as probable:

1. **Village units**
These are indicated for villages that usually produce green stuff, where the growth of lucerne and other green crops is possible for they are well adapted to the socio-economic circumstances:

- They create employment, the process involves 3 people; farmers provide the crop,
- A co-operative basis is possible,
- The simple equipment can be made locally,
- The byproduct (fibrous residue) is used by animals (especially suitable for rabbits),
- The yield of LC (fresh or dried) is low (4 – 5 kg DM/day), but that is enough to supplement 400 – 500 diets.

The activity generated creates an economic micro-system: fields, harvest, production and marketing in which each person can find motivation and reward.

2. Industrial production

To supply large centres of population and dry zones that need greater quantities of LC, it would be necessary to set up small factories in fertile irrigated areas, such would require:

- a zone supplying the crop, organized with the farmers (eventually, perhaps, as a co-operative),
- a small extraction factory, simple, robust and easy to operate, producing dry LC which might be ready-mixed or not with flours, or in ready-to-eat local foods; as well as the fibrous residue for incorporation in animal rations.

Thus one would have a small industrial enterprise, creating numerous jobs and assisting regional food security with LC, together with a small feed industry able to stimulate local stock-breeding.

2.3. Production of LC in France

The development of the consumption of LC is unlikely to come without help and cooperation from the developed countries, especially France. In the early stages substantial outside supply of LC would be needed for its introduction and promotion or to meet local needs. There are 3 situations for which French production is essential:

1. Promotion of LC followed by development of its use

For acceptance, LC has to show its ability to reduce or prevent the consequences of malnutrition. Initially, the medical authorities have to be convinced -especially he health workers involved in supplementation programmes- and also the political and economic decision-makers. The best way to do this is by demonstration through medically monitored nutritional trials and popularization by distribution to canteens, orphanages, etc.

Several years may elapse between the starting of such programmes of persuasion using LC from France and arrival at self-sufficiency.

2. Emergency intervention

In case of war, political trouble, drought or bad harvests, the international community and NGO usually intervene with cereals taken from world stocks. LC is an excellent complement to these seeds. It is easily moved and stored. It is conceivable that a stock of LC should be set up for use in such emergencies, pending restoration of normal supplies.

3. Palliative intervention

There are regions where, for various reasons (lack of organization, great poverty, extremes of climate) self-sufficiency will take decades while millions are undernourished. There, a long term intervention will be needed.
In the world, at present, only 3 factories of the FRANCE LUZERNE Group are producing LC: 12 000 tons/year for animal feed. If all the present French crop-dryers factories were to be similarly equipped, production would amount to about 100 000 tons. That would meet the need of 30 million people (on the basis of 2.5 – 3.5 kg/year/head). There are at least 2 billion undernourished in the world.

4. Perspectives for the French industry

It could be that the value added by the production of LC might encourage the establishment of new plant, whereas the standard crop dryers are at present uneasy and uncertain about their future.

As for the setting up of plant in developing countries, France could have an important role as FRANCE LUZERNE is the only producer in the world. This company could bring its experience and know-how to bear. Where appropriate, financial participation in joint enterprises with local entrepreneurs is envisaged.

Ultimately, it is probable that LC will be of interest in developed countries by entering the vigorously growing markets for nutritional complements and nutritional therapy.

GENERAL CONCLUSIONS

The world's green vegetation presents an enormous mass of leaves of incomparable richness, containing more nutrients than grain or roots.

Their protein value equals that of most animal products and surpasses them for vitamins and trace elements.

A cheap solution would be to eat more leaves, but man's digestive system is unable to digest and absorb a sufficient volume of leaf to match his nutritional needs.

To get round this problem of volume, our association suggests extracting from it the most valuable nutrients, proteins, vitamins and trace elements, removing the indigestible fibrous part and thus to make them available, as a dry concentrate (LC), to supplement the diets of the malnourished.

LC already exists. For 20 years it has been used for particular purposes in animal nutrition. France is the only country in the world that makes it on an industrial scale as dry concentrate: 12 000 T of lucerne LC a year. On the other hand, an English Charity, Find Your Feet, has pioneered, in several Asian and Central American countries, LC extraction from local foliage in simplified forms. These products have already produced very satisfactory results reported in several medical journals.

The French technique for making dry LC lets it keep for over a year with consistent and more or less uniform nutritional value. The first results, obtained in Romania, Nicaragua and China, confirm the nutritional value. Although optimal conditions could not be achieved for pursuing the experiments, due to insufficient finance, all the trials agreed in observing: improvements in state of health, fewer days of sickness, increased levels of Hb, absence of side-effects.

This new approach to nutritional supplementation would seem, more and more, to offer an effective and lasting solution to the imbalanced diets of families or populations in difficulty. Thanks to its richness in lysine, tryptophan, iron, calcium and vitamins, LC restores the balance of diets based on grains. It improves growth and resistance to illness. In very young children it allows normal brain development, thus safeguarding their ability to learn and their chance of a better life.

It can be an alternative to foods of animal origin as well as fruit and vegetables. It is:

- Relatively simple to make, distribute and use,
- Very cheap: 30 FF (or 5 US$)/child/year,
- Of exceptional nutritional value,
- Suitable for replication world-wide,
Dependant on enormous and inexhaustible supplies of leaf.

LC has been shown to be a new food source of great value for man. It deserves careful attention and official recognition in medical circles and by the Food Directorate of the Ministry of Agriculture.

Jacques SUBTIL
Président of APEF
**ANNEX I**

Personalities in the medical & scientific fields who lend their support to the LC project

In tribute to:

the late Professor Henri LESTRADET  
A strong supporter of the project, who died in July 1997,  
Former Member of the National Academy of Medicine,  
Former President of the French Nutrition Society.

**APEF’s scientific advisors**

Professor Julien COLEOU  
Member of the Academy of Agriculture,  

Professor Jean-Claude DILLON  
Former director, ORSTOM nutrition, Dakar,  
Professor of nutrition at the Institut des Cordeliers, Paris.

Doctor Christian RECCHIA  
Nutritionist  
President of the « Centre des Etudes et de Perspectives sur les vulnérabilités humaines ».

**University Hospital Centre (CHU) at Reims**

Professor Jean-Claude ETIENNE  
President of the « CME du CHU »  
Member of Parliament for the Marne  
Vice-president of the Regional Council.

Professor Serge KOCHMANN  
Formerly Senior Member of the Faculty of Medicine  
General councillor – Deputy-mayor of Reims.

**Members of the CHU Steering Committee for LC**

Professor Marc LEUTENEGGER  
Head of Department of Diabetics/nutrition  
Co-ordinator of the Steering Committee.

Professor Henri CHOISY  
Director of Pharmaco-Toxicological Laboratory.

Professor Philippe BOUCHET  
Botany Laboratory, Faculty of Pharmacy.

Doctor Eric KARIGER  
Public Health – Head of the LC project.

**ROMANIA (group of experimenters)**

Professor Corneliu ZEANA  
Head of Cardiology Department, Bucarest Emergency Hospital.

Professor Targoviste IONESCU  
Head of Diabetic Department.

Doctor Gabriela ZEANA  
Head of Research in Industrial Medicine.

**NICARAGUA (Scientific Committee of SOYNICA)**

Professor Josefina BONILLA  
Public Health – Faculty of Medicine, Managua.

Doctor Petronila TERAN  
Paediatric Nutritionist,  
Medical supervisor of the SOYNICA project.
ANNEX 1 (Concluded)

CHINA (Agricultural University of China, Beijing)

Professor GUO Peiyu  President of the Chinese Leaf Protein Preparatory Association,
Former Vice-president of the University.

Professor HAN Lujia  Head of the Institute for Unconventional Foods.

Professor LIU Xiangliang  Institute for unconventional foods.

Professor DENG Yong  Food Science.

ITALY (University of Perugia)

Professor Paolo FANTOZZI  Director of the Institute of Agricultural Industry at the University of Perugia,
Chair of Food Technology.

Doctor Luigi MONTANARI  Biotechnologist.

(the University of Perugia collaborates with APEF on the CHINA project).
# ANNEX II

## ANALYTICAL METHODS

employed for nutritional and toxic materials

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>REFERENCE METHOD</th>
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<tbody>
<tr>
<td>Moisture</td>
<td>CEE 2\textsuperscript{nd} Directive TI/393</td>
</tr>
<tr>
<td>Protein (Dumas)</td>
<td>AFNOR V18 –120 (March 1997)</td>
</tr>
<tr>
<td>Protein (Kjeldahl)</td>
<td>CEE Directive 93/28</td>
</tr>
<tr>
<td>Fibre (Cellulose)</td>
<td>Weende's method (AFNOR VO3-040 &amp; CEE Directive)</td>
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<tr>
<td>Crude Ash</td>
<td>AFNOR NF V18-101</td>
</tr>
<tr>
<td>Fats</td>
<td>CEE 2\textsuperscript{nd} Directive, Process A</td>
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<tr>
<td>Carotene</td>
<td>AOAC 1980-43.018, modified</td>
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<tr>
<td>Carotene-Xanthophylls</td>
<td>BIPEA n° AC 81.M 85.10</td>
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<tr>
<td>Vitamins A &amp; E</td>
<td>AFNOR NF V18-402 (1997)</td>
</tr>
<tr>
<td>Amino-acids</td>
<td>AFNOR NF V18-113 (except tryptophan)</td>
</tr>
<tr>
<td></td>
<td>AFNOR V18-114 (Tryptophan)</td>
</tr>
<tr>
<td>Minerals</td>
<td>CEE 2\textsuperscript{nd} Directive &amp; NF V18-106 (Phosphorus)</td>
</tr>
<tr>
<td></td>
<td>NF V18-108 (Calcium)</td>
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<tr>
<td></td>
<td>CEE 8\textsuperscript{th} Directive (Copper, Manganese, Zinc &amp; Iron)</td>
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<td></td>
<td>CEE 4\textsuperscript{th} Directive (Magnesium)</td>
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<tr>
<td></td>
<td>CEE 1\textsuperscript{st} Directive (Sodium)</td>
</tr>
<tr>
<td>Aflatoxins</td>
<td>AOAC, modified by HPLC</td>
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<tr>
<td>Bacteria</td>
<td>NF ISO 4833, July 1991 (Micro-organism counts)</td>
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<td></td>
<td>NF VO8-017, June 1980 (Fecal coliforms, E. coli)</td>
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<tr>
<td></td>
<td>NF VO8-019, December 1985 (Clostridium perfringens)</td>
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<td></td>
<td>NF VO8-014, January 1984 (Staphylococcus aureus)</td>
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<td></td>
<td>NF ISO 6579 (Salmonella)</td>
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<tr>
<td>Heavy metals</td>
<td>Internal method of the Laboratory of Hygiene &amp; Public Health Research – Faculty of Medicine – Nancy.</td>
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<tr>
<td></td>
<td>Wet mineralization in HNO3 and for H2SO4 analysis by mass spectrometry.</td>
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<td>Pesticides</td>
<td>Internal method of the Laboratory of Hygiene &amp; Public Health Research – Faculty of Medicine – Nancy.</td>
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<td></td>
<td>Organochlorines by Gas Chromatography and Electron Capture detection.</td>
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<tr>
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<td>Organochlorines by Gas Chromatography and Nitrogen &amp; Phosphorus detection.</td>
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</tbody>
</table>
ANNEX III

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