

## METHODS AND MERITS OF FODDER FRACTIONATION

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Leafy crops have, in many climates, no rivals in productivity. That is to say, when given adequate amounts of water and fertiliser they give a larger annual yield of dry matter (DM) per ha than crops that divert their product from the leaf into a seed or underground part. If this potentiality is to be fully exploited, the crop must be harvested when it is still in a physiologically active state. After harvest many species will regrow: those that will not must be resown. In either event the ground soon has a young and active photosynthetic cover rather than material that is merely maturing and translocating. The great productivity of leafy crops depends on this periodic rejuvenation.

Green vegetables are harvested when physiologically active, and they are perishable both when taken young and when nearing senescence. The additional problems raised in their distribution if they are harvested when they contain 92% of water rather than 84% are not formidable. That difference in water content means, however, that the purchaser gets only half as much DM in the one vegetable as in the other and usually pays more for it. This is an important point that will not be properly appreciated until those concerned with market gardening have been persuaded to publish figures on the quality and yield of their product that resemble those published by other food producers. It is at present very rare to find published yields from which the amount of protein and DM produced annually from a vegetable crop can be calculated. There is, however, good reason to think that in many regions the annual yield per ha, of material directly edible by people, is greater from market gardening than from any other method of using land.

Large as the yields of garden produce are, they are not so large as the yields attainable with forage crops. In countries where growth stops in winter, the annual yield can be 20 t DM per ha; in the tropics it can be 40. For as long as forage crops are thought of simply as ruminant fodder, their potentialities will not be fully exploited because only 5 to 15% of the protein in ruminant fodder is returned in edible form in meat and milk, and 3 to 10% of the energy. The amount of human food produced is therefore greater when land is used to grow cereals for energy, and seed legumes for protein, in spite of the smaller yields of DM from these crops.

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Professor Norman W. Pirie, F.R.S., is well known for his work on various aspects of biological sciences, especially on viruses, on separation and properties of macromolecules, and on biochemical engineering with special emphasis on methods for increasing world food production.

The production of almost all food involves some fractionation. Cereals are separated from chaff; potatoes are peeled; sugar is extracted from the cane or, when cane itself is chewed, the pith is not swallowed after being chewed; the petioles and outside leaves of many vegetables are rejected. In view of the last example of separation, or selection, it is odd that it has taken so long for the idea of fractionating forages to gain acceptance. Early work on fractionation, in Britain at any rate, depended on the greater rapidity of drying of the leaf lamina compared to the petiole and stem. After partial drying and light milling, the laminar protein-rich part of the leaf will pass through a sieve that holds back the moist and less broken fibrous part. In this way it is easy to separate a fraction with twice the nitrogen (N) content of the original crop. This fraction would be a useful source of protein and carotene for pigs and poultry and could be eaten in small quantities by people. The starting material for this type of fractionation should be a protein-rich broad leaved crop; this is likely to contain 90%, or even more, water and would therefore be expensive to dry.

To take full advantage of the potentialities of leary crops, they must be harvested when they are lush and growing vigorously. The product, as harvested, needs some form of preservation unless it is used within a few days. In most climates in which herbage grows well, sun-drying cannot be relied on. For that to be reliable, the crop must be allowed to grow to maturity; it then becomes hay and the annual yield is diminished. A lush crop can be preserved by ensiling or drying. Drying is less expensive if part of the water is evaporated by field-wilting, possibly after crimping, crushing or assiduous tedding. These processes are wasteful because part of the shattered crop will not be ultimately raked up, and because there will be serious loss of soluble constituents (e.g. sugars and N-containing substances either present initially or formed by protein autolysis in the dying leaf) if it should rain. Several attempts were at one time made to press out the water. Very little can be pressed from an undamaged leaf. What success these attempts had depended on the inefficiency of the pressing technique; the rollers or screw expellers rubbed the leaves in the course of pressing them and so released juice from damaged cells. The juice thus liberated brings out with it the soluble components (including protein) of the leaf.

A lush crop can be used in four ways: each has advantages and limitations:—

There is so much damage by treading when a lush crop is grazed, that it is better to harvest it even when it is all used as fodder. This 'zero grazing' is satisfactory during the growing season, but there are serious losses when silage is made.

Where fuel is abundant the crop can be dried for winter feeding. This wastes a great deal of energy as latent heat of evaporation when water vapour is discharged but it would in principle be possible to make the process more economical by using some form of 'vapour compression' so that liquid water is discharged. It will be difficult to feed the crop into the necessary vacuum chamber except in a batch process.

Although little juice can be pressed from an undamaged leaf it is easy to press juice from leaves that have been heated to about 70°C. Unless pressing is accompanied by disintegration, the protein remains in the leaf fibre, but sugars and non-protein N come out

in the juice. This therefore is a form of fractionation and it would not be economic unless some use is found for the juice. I will discuss possible uses later. After pressing for a few seconds on a suitable grid to 2 or 3 kg cm<sup>-1</sup> the fibre contains 70% water. This would have to be dried off but it would be necessary to evaporate only 2.3 t of water for each t of DM compared to 7 to 9 t if the original crop had been dried. Fibre containing only 60% water, i.e. 1.5 t for each t of DM can be made by longer and more intense pressing. The limit to water removal by pressure alone is about 45% DM. If 1 t of material with that composition were made by pressing fibre that initially contained only 30% DM 0.5 t of fluid would be expressed. The amount of work needed for the pressing would be < 1MJ whereas it would take > 1000 MJ to evaporate that amount of water.

The advantage of fractionating a crop in that way is that much less fuel would be needed to dry the fibre for use as winter feed. But it would be suitable only for feeding ruminants and they do not need to take in all their food N in the form of true protein; part of the protein can be replaced by simple N compounds such as urea which are converted to protein by the rumen micro-organisms. There is therefore often no advantage in retaining as much protein as possible in the fibre. Furthermore with intensive fertilisation, irrigation and regular cutting so as to get the maximum yield the herbage would contain 20 to 25% protein which is an unnecessarily large concentration for ruminant feeding. Material of this sort would be a useful supplement to protein-deficient fodder such as straw but it would be wasteful to use it as a main foodstuff. It seems therefore that if herbage is being harvested, transported to a processing unit and put through a fairly elaborate machine, it would be advantageous to separate as much protein as possible from it and use only the protein-depleted fibre as ruminant fodder. In the laboratory, separation can be nearly complete; it would be impractical in large scale practice to aim at more than 60 to 70% separation. The protein fraction, like the fraction already mentioned, made by milling partly dried leaf, could be used as feed for non-ruminants. Unlike that fraction, it contains so little fibre that it could also be used in quantity as human food. By definition, people use dietary protein with 100% efficiency, and non-ruminant animals, e.g. pigs and poultry, are more efficient than ruminants at converting fodder protein into human food.

To release protein from many types of leafy fodder it is not necessary to grind the leaves finely—thorough rubbing is sufficient. That is why rollers and screw expellers which are designed to crush and press release some of the protein. Hitherto we have rubbed the crop in pulpers with beaters running at tip speeds of 2 to 6 m/s<sup>-1</sup> and have pressed juice from the pulp in a separate operation. Equipment for doing this on various scales is described in a book published by the International Biological Program<sup>1</sup>. With this equipment many hundred t of forage have been processed in Britain and elsewhere, but the arrangement is far from ideal: too much power is wasted in creating wind. It would be convenient to make extraction a unit process. I have suggested<sup>1</sup> a design for a unit that would both pulp and press its principle is illustrated in Fig 1.

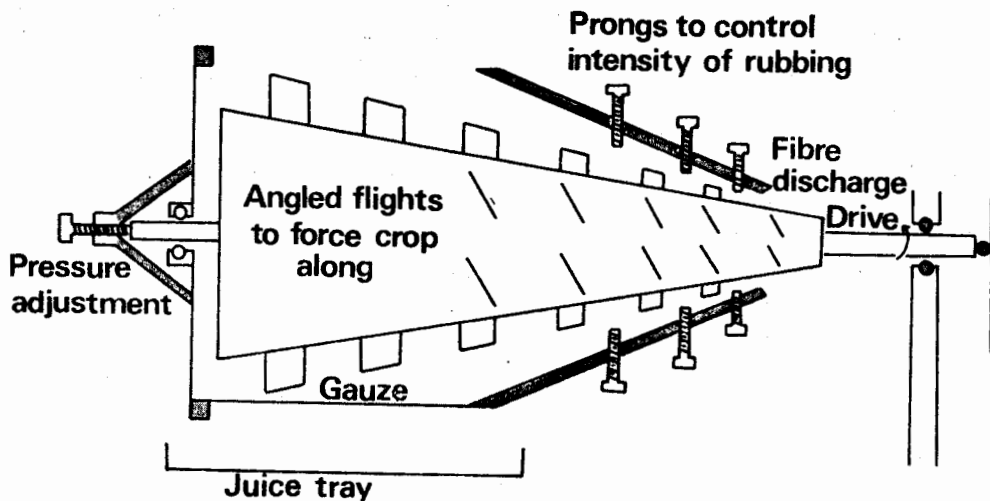


Fig. 1

Experience with other types of equipment suggests that as soon as there is a packed mass of leaf fibre in the narrow part of the annulus, juice will flow back and out through the gauze. Retained particles will not block the gauze because it will be continually wiped by the incoming crop. The underlying principle in this suggested design is that the volume in which most of the work on the crop is done should be kept small. This ensures that contact between fibre and released juice will be brief; prolonged contact leads to loss of extractable protein.

This arrangement differs from a conventional screw expeller in that there should be little useless friction—the mere sliding of the surface of the scroll over the charge—and because juice is not separated from the pulp under pressure less fibre will be pressed through into the juice. The arrangement differs from a 3-roll 'sugar cane mill' in that the action is a rubbing rather than a crushing and juice is allowed ample time to flow out of the unit. In a 3-roll mill much of the juice is reabsorbed by the fibre after passage through the 'nip' because unless the mill is run extremely slowly pressure is maintained so briefly that the juice has insufficient time to flow away from the compressed fibre.

The intensity and duration of the pressure that should be applied to pulped fibre depend on the objectives of fractionation. If the fibre is later to be ensiled and if maximum protein extraction is not required, 3 to 5 s at 1 to 2 kg cm<sup>-2</sup> in a belt-press of the type described by Davys and Pirie<sup>2</sup> is sufficient. That will remove enough juice to prevent any loss in drip from the silo however wet the original crop. When maximum protein extraction is required pressure should be maintained for 5 to 10 s at 2 to 3 kg cm<sup>-2</sup> and the pressed material should afterwards be mixed intimately with water and pressed again without delay. More intense and prolonged pressure brings out more juice but this contains little protein. As already mentioned this more intense pressing is advantageous if the fibre is to be dried.

The extracted juice can be used in its original state for feeding pigs or as a milk replacement for calves. Its composition depends on the protein and water content of the crop—the dry weight of true protein in it can vary from 1 to 10% of the wet weight of the juice. Within a few hours or days, depending on temperature, most of the protein coagulates. Coagulation is immediate if the juice is heated or if acid is added. The sediment that then settles out varies in bulk but has a fairly constant composition—15 to 20% of the wet weight is protein. This wet sediment would seem to be the most suitable material for use with pigs or calves, partly because of its greater uniformity, partly because less formic, phosphoric or propionic acid will be needed to preserve it, and partly because, with pigs at any rate, the non-protein N and polysaccharides in the supernatant fluid are of questionable nutritional value.

Heat coagulation is the ideal method for making a protein curd for use as human or poultry food immediately or after preservation. Its merit depends both on the inactivation of enzymes and on the formation of a dense coagulum that is easy to separate from the liquor or 'whey'. Enzyme inactivation is important with crops, such as lucerne (*Medicago sativa*), rich in chlorophyllase. If that enzyme is allowed to hydrolyse chlorophyll to chlorophyllide the latter may be converted to pheophorbide (by loss of magnesium) which can make animals photosensitive<sup>3</sup>. These changes are prevented<sup>4</sup> by injecting steam into the stream of leaf extract so as to heat it quickly to 100°C. When coagulation, rather than enzyme inactivation, is all that is required, it is sufficient to heat to 70°C, but quick heating remains advantageous. It is also advantageous to separate the curd from the 'whey' quickly to minimise combination of the protein with phenolic substances in the extract. Standard equipment, used to separate such materials as yeast, dyestuffs and sewage sludge is suitable. Material intended as human food should be pressed so as to separate as much 'whey' as possible from the curd; the curd should then be suspended in water at about pH4 and filtered off again. This removes most of the leaf flavour and makes the second filtration easier. Obviously, as much 'whey' as possible should be pressed out each time so as to increase the effectiveness of washing. The pressed curd contains 60 to 65% of water; it can be preserved like any other lipid-containing protein by canning, drying, pickling or the addition of salt. As Rouelle<sup>5</sup> observed two hundred years ago, carefully controlled heating coagulates that fraction of the protein which is associated with chlorophyll, if this is removed, nearly colourless protein can be separated by heating the fluid to a higher temperature. It is also possible to sediment all the chlorophyll-containing protein by centrifuging unheated juice at > 20,000 relative centrifugal force (e.g. ref. 6), but such a procedure is not likely to be feasible in large scale practice.

Protein made from many species, without separation of the green and white fractions, contains 9 to 11% N. The green fraction contains 1 or 2% less, and the white fraction 1 or 2% more N than the unfractionated material. Some species consistently yield products containing less N than that. If juice is properly stained after pressing from leaf pulp, the coagulum from it will contain less than 1% of fibre. From some species, notably pea haulm (*Pisum sativum*) taken as a by-product from canning or freezing, it contains as much as 10% of starch. However, the main reason for the N content being less than would be expected in a protein is the presence of 15 to 25% of highly unsaturated lipid<sup>7-9</sup>. The

lipid is easily removed by solvent extraction, but this removes  $\beta$ -carotene also; carotene and unsaturated lipids are valuable components of animal or human food. Many people advocate solvent extraction because it removes the chlorophyll and they think that a product that is not green will prove more acceptable. My experience is that an unfavourable reaction to the green product passes off in 1 or 2 weeks. If surface dust is removed from the crop by spraying with water before pulping, and if the coagulum is properly pressed and washed, it should contain <1% of material soluble in water, <3% of ash and <1% of acid-insoluble ash. Even when allowance is made for the presence of fibre, starch, lipid and ash, leaf protein (LP) preparations usually contain less N than would be expected. The results of Allison *et al.*<sup>10</sup> suggest that this is in part the result of fixation of phenolic compounds by amino groups in the protein. When the  $\epsilon$ -amino group of lysine is involved, this combination is nutritionally detrimental. There are great differences in the amounts of phenolic material present in varieties of the same plant species; when herbage is to be fractionated to produce LP it will probably be worthwhile searching for varieties relatively free from phenolic compounds.

Amino acid analyses on LP preparations made recently from several different species of leaf are similar<sup>11</sup> and confirm the conclusion reached many years ago that LP should be valuable nutritionally. This conclusion is amply confirmed by feeding experiments on animals<sup>12</sup> and children<sup>13</sup>. As would be expected, LP is surpassed by casein and egg protein but is as good as fish protein and better than any seed protein that is available in bulk.

The composition of the 'whey' pressed from the curd after heat coagulation depends on the weather at the time of harvest, the species that is processed, its stage of growth, and the amount of water that remains on the herbage after washing dust from it. The ranges in analyses<sup>14</sup> on 61 samples were: DM 11 to 47 g l<sup>-1</sup>, carbohydrate 2.2 to 22 g l<sup>-1</sup>, and N 0.25 to 1.2 g l<sup>-1</sup>. Carbohydrate soluble in 80% ethanol, i.e. sugars rather than polysaccharides, was 49 to 90% of the total carbohydrate. The 'whey' contains about 15% of the DM of the crop; a larger fraction of the DM is in the 'whey' from young than mature crops, and a larger fraction of the N is in 'whey' from legumes than from other species. Most of the K and much of the P of the leaf is also in this fraction but there have been no systematic analyses. The simplest way to dispose of the 'whey' is to sprinkle it back on an area of ground similar to that from which the crop came. The NPK in it would be useful and on some soils the carbohydrate in it stimulates bacteria that improve soil structure<sup>15</sup>.

Where there is a regular and abundant supply of 'whey' it will be used as a microbial culture medium. Some early experiments with it are summarised in the IBP Handbook<sup>1</sup>. Worgan<sup>16</sup> estimates that the 'whey' from single harvests of 1 ha of maize (*Zea mays*) or pea haulm would yield 110 and 125 kg of microbial protein respectively.

The main reason for fractionating a fodder is that, if some fractions are used to feed non-ruminant animals, more human food is produced than ruminants would have produced from the whole crop. Obviously this merit is enhanced if a product is made with sufficient care for it to be used as human food. These points are illustrated in Fig. 2.

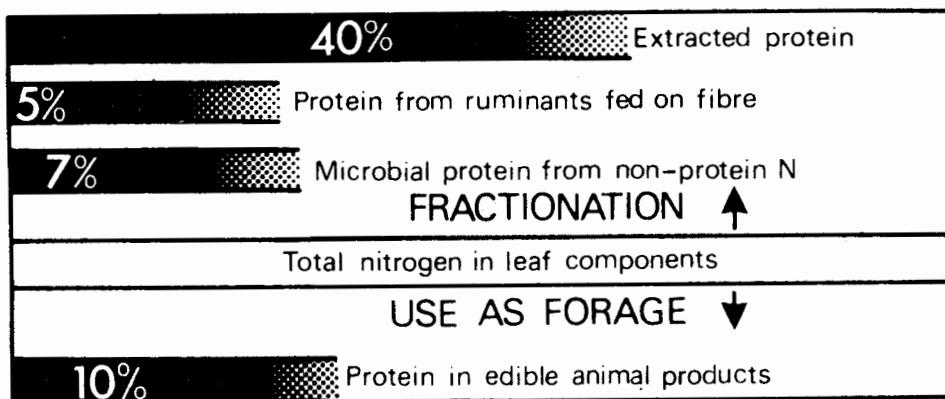


Fig. 2

Increased efficiency is a real merit even when the herbage is grown in the traditional manner but there would be little advantage in departing from tradition if the method of fractionation does not separate the protein from the fibre but leaves it in a form suitable for use as ruminant fodder only. If much of the protein is separated from the fibre farmers are given an incentive to produce herbage that is unnecessarily protein-rich for feeding ruminants and when this is done the total yield is increased. Therefore fractionation not only increases the value of existing crops, it also provides a practical incentive to produce better crops. When the quality, i.e. the protein content, of herbage is improved by frequent cutting, the annual yield of total DM is usually diminished. When quality is improved by increased use of fertilisers and judicious irrigation, the yield of DM is increased. For brevity, attention will be restricted here to the yield of extracted protein.

As I have mentioned extracted LP contains 9 to 11% N. To ensure comparability all results will be expressed as N x 6, i.e. in terms of approximately 100% protein. Results can be expressed either as protein yield per ha in a year, or as the rate of production per day. The former method under-values the rate per day in temperate compared to tropical climates because there is so little growth in winter; the latter method may over-value the rate unless it is borne in mind that the rate stated may not include a period for the establishment of a sward, and that it may not be possible to maintain the stated rate throughout a year even in the tropics. None of the values should be thought of as limits. We are still learning a new form of agronomy; equipment, and therefore the percentage of the total protein that is extracted, is still being improved; and the species used as sources of LP were developed by plant breeders for entirely different purposes. Fig. 3 illustrates this. It is a 'learning curve' showing our increasing skill at Rothamsted and there is no reason to think that the limit has been reached. These were field yields without irrigation; the values for summer rainfall suggest that the apparent ultimate flattening of the curve is merely the result of dry summers. The yields given in Fig. 3 were from various crops grown in succession on the same piece of ground, irrigated ryegrass (*Lolium perenne*) gave 1.8 t ha<sup>-1</sup> at Rothamsted and unirrigated gave 1.2 t in New Zealand<sup>17</sup>. Allison and Vartha<sup>18</sup> got 2 t ha<sup>-1</sup> from irrigated lucerne in New Zealand; Dev *et al*<sup>19</sup> got 3.1 t ha<sup>-1</sup> from it in a dry part

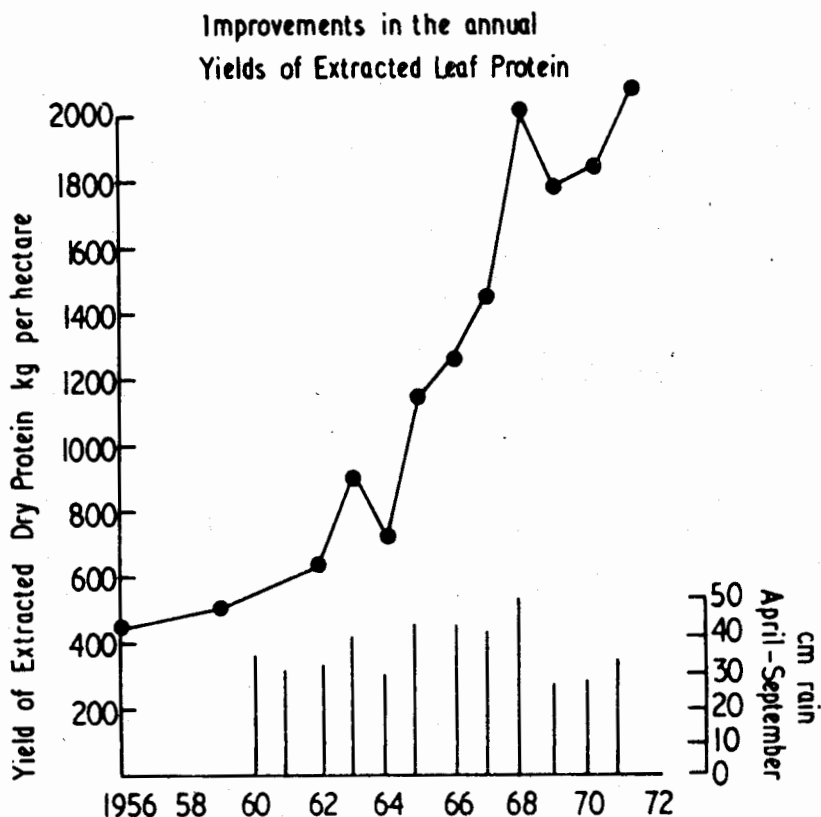


Fig. 3

of India. In the same region, irrigated hybrid Napier grass (*Pennisetum purpureum* X *P. typhoideum*) gave 2.25 t ha<sup>-1</sup> although only 30% of the protein was being extracted<sup>20</sup>. Several species less amenable to regular cutting than lucerne and the grasses, notably cowpea (*Vigna unguiculata*), give similar or even greater rates of synthesis of extractable protein in Indian conditions<sup>21,22</sup>.

Considering these and other results it is reasonable to conclude that improvements in agronomy, extraction technique, and choice of plant species and variety, could increase the annual yield of extracted 100% dry protein to 3 t ha<sup>-1</sup> in temperate climates and to 5 t ha<sup>-1</sup> in the tropics. Whether it will be practical to strive for such extremely large yields is uncertain. They depend, even when leguminous species are used, on liberal use of fertilisers and it is well known that, although leafy crops usually make use of a larger proportion of the applied fertiliser than other crops, the greater the amount of fertiliser used, the smaller the percentage of it that ultimately appears in the crop. This is a matter for economic argument and for discussion with those concerned with the contamination of streams coming from farmland.



Many factors control the selection of herbage for fractionation. Leguminous crops have obvious advantages although large yields cannot be maintained by reliance on their root nodules as the sole source of N. Leaves that are the by-product of a conventional crop should be used whenever possible. In Britain we get from sugar beet tops 600 kg ha<sup>-1</sup> and from main crop potatoes 300 kg ha<sup>-1</sup> as well as 2 or 3 t of fibre containing 1.5 to 2% N. From early potatoes the yield of extracted protein is greater. There are potentialities in the leaves of such crops as jute (*Corchorus* sp.) and ramie (*Boehmeria nivea*). Surplus leafy vegetables and their outer leaves, e.g. those from cauli-flower (*Brassica oleracea* var. *botrytis*)<sup>23</sup> should be a useful source of LP because their disposal is sometimes expensive at present, but nothing would be gained by extracting protein from a leaf that could be eaten in the conventional manner. Mixed weeds growing on waste land are not a likely source; after being cropped once or twice the land would have to be fertilised, if that is being done and if the land allows mechanical harvesting, it would be better to sow it with a more desirable crop. By contrast, water weeds have potentialities<sup>24</sup>: they would be easy to process on barge-mounted equipment, they are usually botanically homogeneous, they are often well fertilised, and attempts to kill them with herbicides are, at present, very expensive. Because LP is washed at pH 4 there is little or no risk that poisonous leaf components will remain associated with it; if their presence in the fibre is feared, it also can be washed with dilute acid.

Scientists realised 50 years ago that the components of leaves would be more useful when separated than when mixed together, and practical separation units were made 20 years ago. The evidence accumulating since then has in the main confirmed the earlier conviction. As a result there is now active research in Britain on the use of forage fractions in the Department of Agriculture of the University of Reading, the National Institute for Research in Dairying in Reading, and the Rowett Research Institute in Aberdeen. There is also active research in Eire<sup>25</sup>, New Zealand, Nigeria<sup>26</sup> and Sweden and there is both research and commercial production in Hungary<sup>27</sup> and USA<sup>28</sup>. Considering the potentialities of herbage production, especially in those parts of the wet tropics where the ripening of a seed crop is uncertain, the amount of research that is being done is totally inadequate compared to what is done on protein sources such as oil seeds and micro-organisms. This is clearly shown by the absence of work, in any country, on the selection of varieties from which, because of structure and the absence of phenolic compounds, protein is readily extracted; by the slow progress in the redesign of extraction equipment; and by the absence of concrete evidence on costs.

In many quarters there is still much scepticism, or even hostility, towards the idea of making LP either as an animal or a human food. This arises in part from reliance on the gloomy conclusions<sup>29</sup> come to 20 years ago on the basis of the poor nutritive value of some inadequately washed and over-heated preparations, and the unacceptability of the silage they made from the fibre. Acceptable silage has been produced by others<sup>1,30,31</sup>. Scepticism also gained support from results<sup>32</sup> that should have been taken as no more than evidence that the authors lacked skill in growing and processing crops. Costs will be guesswork until the continuous operation of an extraction unit can be studied carefully; even when total costs are known, the manner in which they, and payments, are distributed

between protein, fibre, and 'whey' will be arbitrary. At a date when our yields were those shown to the left of Fig. 3, and when the power consumed in extracting a kg of protein was 3 times what it is now, FAO stated that LP would be too expensive to be useful. FAO continues to assert this and so does its Protein Advisory Group. Similarly, thinking exclusively of European and North American food habits, these organisations think LP unacceptable. This is not the opinion of those with experience of India and West Africa, nor of many of those in Britain with experience of LP lasting more than a week. A reassessment of the facts seems overdue.

In spite of the last paragraph, this paper is not intended primarily as a re-presentation of the case for LP. I have taken the opportunity to update the IBP Handbook on the subject—most of the argument is presented there. The object of this paper is rather to suggest that with herbage, as with sugar beet, the material harvested should be looked upon as the input to a fractionation process. Because herbage is more perishable than sugar beet, leaf processing units would seldom, if ever, be as large as sugar beet factories, and there is more scope for varying the extent of extraction and the uses to which the products would be put. But the underlying ideas are similar.

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