PRODUCTION OF EDIBLE PROTEIN FROM

PERSIAN CLOVER

(Trifolium resupinatum)

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These studies were carried out in the Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories, Lahore during the period from November 1969 to January 1974. The author undertakes that the research work presented in this thesis has not been submitted to any other University or Institute for the Degree of Doctor of Philosophy and shall not in future be submitted for obtaining similar degree of any other University.

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DATA ENTERED
ABSTRACT

Persian clover (Trifolium resupinatum) was cultivated on a soil which was analysed completely by mechanical and chemical methods. The changes in atmospheric temperature and relative humidity during the growing season were recorded. The crop was harvested after different intervals and the effects of climatic conditions, length of day (photosynthesis period) and age on the crop were studied.

The plant growth, dry matter and nitrogen content varied considerably during the season due to the changes in atmospheric temperature, relative humidity, length of day and age. Harvesting of the crop after an interval of 20-100 days showed variations in yield/hectare of fresh and dry weights and nitrogen content in a season. Thirty day old 1st cut and the successive regrowths gave the maximum yield of fresh and dry weights, i.e. 86.59 and 11.23 ton/hectare/season. The total nitrogen content was however, more in case of 20 day old 1st cut and regrowths, i.e. 42.3 kg/hectare/season.

The amount of extractable nitrogen (EN) in the juice and extractable protein nitrogen (PN) precipitated
from the juice by heating at $80 \pm 2^\circ C$ or with TCA, was also affected considerably by the changes in climatic conditions, age and maturity of the crop. The percent extraction of these fractions varied negligibly up to the age of 60 days, till the end of March. The per hectare yield of EN and PN however increased. Twenty day old 3rd regrowth, 30 day old 3rd regrowth, 40 day old 2nd regrowth and 60 day old 1st regrowth, harvested in the month of March, gave comparatively more yield hectare of EN and PN than that of their respective 1st cuts and regrowths. The percent as well as per hectare yield of these fractions continuously decreased in 20-100 day old regrowths taken after March, when the flowering and seed development was in progress. The maximum yield of easily extractable protein in a season was 900 kg/hectare, that was obtained from the 20 day old 1st cut and subsequent eight regrowths.

The percentage of non-proteinous nitrogen (NPN) in the juice increased with an increase in the age of plant and such changes were more rapid during reproductive stages. The over all variations in NPN during the season were from 13.2 to 11.0% when the age of 1st cut and regrowths increased from 20-100 days. This increase in NPN was attributed to the breakdown of proteins by
the proteolytic enzymes present in the plant.

The percentage of FR increased and its nitrogen content decreased with an increase in the age of cuts and regrowths. The per hectare yield however, depended on the yield of crop. Thirty day old 1st cut and regrowths gave maximum yield of FR (6.412 Ton/hactare/season) which contained about 200 kg. nitrogen. Twenty day old 1st cut and regrowths although contained the same amount of nitrogen but the yield of FR was comparatively less, viz. 5.131 Ton/hactare/season. The FR obtained from 20 and 30 day old cuts and regrowths contained sufficient proteins (17.5 and 20.7%) and can be recommended for animal feeding.

The volume of waste liquor and its nitrogen content depended entirely on the volume of the juice extracted from the crop and its NPN content. The yield of waste liquor from 20 to 30 day old harvests (1st cuts and regrowths) was from 6459.2 to 67338 litre/hactare/season. This volume was much more than that of 60-100 day old 1st cuts and regrowths. This by-product contained considerable amount of nutrients and proved to be a suitable substrate for propagation of Candida utilis. The yield of food yeast on the dry weight basis was 5.3 g/litre of waste liquor.
The maximum yield of protein, from a unit area of the land, including the protein obtained from the waste liquor by propagation of food yeast was estimated to be 1078 kg/hectare/season.

The Leaf Protein Cake (LPC) prepared from the crop was dehydrated and dehydrated for increasing the stability or the shelf-life of the product. Several preparations were made from LPC by freeze drying, oven drying, roller drying and solvent extraction. The effects of different processing conditions on the nutritive value of proteins were studied by in vitro and in vivo methods and microbiological estimation of total and available amino acids. The dehydration of LPC by freeze drying and solvent extraction did not affect the nutritive value of the proteins. Drying of LPC over hot air at 70-100°C in an oven or by passing over steam heated rollers reduced the extractability of lipids. Moreover, the in vitro digestibility with papain, net protein utilization and amino acid availability also decreased considerably. This decrease in the nutritive value of heated leaf proteins can be attributed to the oxidation of unsaturated lipids and formation of complexes with the proteins.

The extraction of heated proteins with chloroform-methanol (2:1) mixture or acetone increased their digestibility with papain from 18.0% to 60.8% and net protein
utilization from 47.0 to 67.5%. This increase in nutritive value showed that solvent treatments eliminated the oxidation and copolymerized products formed during moist heating of proteins. The solvent extraction also resulted in a greater release of bound amino acids with papain. None of the bound lysine was released from the heated products with solvent extraction and the amount available to Leuconostoc mesenteroides after the enzymic hydrolysis ranged from 3.81 to 5.0 g/16 g.

The leaf protein cake, products dried over hot air at 80 ± 2°C and the freeze-dried sample after extraction with acetone showed better papain digestibility, APU and amino acid availability than the other preparations. These products can be stored at room temperature and are recommended for supplementing the protein deficient diets of human beings.
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CONTENTS

Abstract ................................................. 1
Acknowledgement ........................................ VI

Page

1. INTRODUCTION ........................................... 1

2. REVIEW OF LITERATURE ................................. 6

3. INFORMATION ABOUT THE PLANT ...................... 21

4. PLAN OF WORK ........................................... 23

5. EXPERIMENTAL ........................................... 24

  a) MATERIAL AND METHODS .............................. 24

     Procurement of seeds ................................... 24

     Preparation of seed bed ................................ 24

     Randomize block design of experimental plots .... 24

     Method of soil description ......................... 26

     Recording of atmospheric temperature and relative
     humidity .................................................. 26

     Seed rate and method of sowing ..................... 27

     Irrigation and crop care .............................. 27

     Intervals between the harvests ...................... 27

     Harvesting and weighing .............................. 28

     Measurement of height and tillering
     ratio of plants ....................................... 28

     Extraction of proteins from crop .................. 28

     Separation of proteins .............................. 29
Propagation of Candida utilis (Food Yeast) on waste liquor............. 30
Drying of leaf protein cake........ 31
Oven drying.......................... 32
Roller drying.......................... 32
Freeze drying......................... 32
Solvent extraction of leaf protein cake.......................... 32
Grinding of samples..................... 33
Extraction of lipids from dried leaf protein concentrate........ 24

b) STATISTICAL........................................... 34
c) ANALYTICAL........................................... 35

Mechanical and Chemical analysis of soil.......................... 35
Estimation of moisture.......................... 35
  " nitrogen.......................... 35
  " proteinous and non-proteinous nitrogen.......................... 35
Estimation of amide nitrogen.......................... 36
  " pH.......................... 36
  " total sugars.......................... 36
  " lipids.......................... 37
In vitro and In vivo digestibilities tests.......................... 38
Microbiological estimation of amino acids.......................... 38
6. RESULTS ................................................................. 40

7. DISCUSSION ON RESULTS ...................................... 100

a) Effects of climatic variations and
age on the yield, dry matter and
nitrogen content of the crop during
the season ................................................................. 100

b) Effects of climatic variations and
age on the yield of extractable
nitrogen, extractable protein
nitrogen and some other fractions of
the crop during the season ................. 107

c) Effects of different methods of
drying and solvents extraction on
the nutritive value of leaf protein
concentrate ............................................................. 118

8. SUMMARY .............................................................. 126

9. BIBLIOGRAPHY ....................................................... 128

10. APPENDIX

Some photographs of Persian clover ....... 136
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Soil description</td>
<td>41</td>
</tr>
<tr>
<td>Table 2</td>
<td>Mechanical analysis of the soil</td>
<td>44</td>
</tr>
<tr>
<td>Table 3</td>
<td>Chemical analysis of the soil</td>
<td>45</td>
</tr>
<tr>
<td>Table 4</td>
<td>Changes in atmospheric temperature and relative humidity during September 1970-August 1971</td>
<td>46</td>
</tr>
<tr>
<td>Table 5</td>
<td>Changes in the height and leaf:stem ratio of plants in the 20, 30 and 40 day old cuts and regrowths</td>
<td>47</td>
</tr>
<tr>
<td>Table 6</td>
<td>Changes in the height and leaf:stem ratio of plants in the 60, 80 and 100 day old cuts and regrowths</td>
<td>48</td>
</tr>
<tr>
<td>Table 7</td>
<td>Fresh weight yield (kg/hectare) of the crop when harvested after 20-100 days intervals</td>
<td>58</td>
</tr>
<tr>
<td>Table 8</td>
<td>Analysis of variance of data from table 7</td>
<td>59</td>
</tr>
<tr>
<td>Table 9</td>
<td>Mean total yields of the crop when harvested after 20 to 100 days intervals including the regrowths</td>
<td>60</td>
</tr>
<tr>
<td>Table 10</td>
<td>Statistical analysis of the data given in table 9</td>
<td>61</td>
</tr>
<tr>
<td>Table 11</td>
<td>Changes in total nitrogen (TN) in the dry matter of pulp, extractable nitrogen (EN) and proteinous nitrogen (PN) in the juice of the crop harvested after an interval of 20 to 40 days</td>
<td>65</td>
</tr>
<tr>
<td>Table 12</td>
<td>Changes in total nitrogen (TN) in the dry matter of pulp, extractable nitrogen (EN) and proteinous nitrogen (PN) in the juice of the crop harvested after an interval of 60 to 100 days</td>
<td>67</td>
</tr>
</tbody>
</table>
Table 13  Changes in percentage of non-proteinous nitrogen (NPN) in the juice of crop harvested after different intervals. 70

Table 14  Effect of different harvesting intervals on the total yield of leaf protein cake (LPC) and its protein content. 82

Table 15  The effects of different harvesting intervals (20-30 days) on the fibrous residue (FR) and its nitrogen content. 83

Table 16  The effects of different harvesting intervals (50-100 days) on the fibrous residue (FR) and its content. 84

Table 17  Effect of different harvesting intervals on total yield of the waste liquor and its nitrogen content. 88

Table 18  Composition of waste liquor (g/100 ml). 89

Table 19  Mineral composition of the waste liquor (mg/litre). 89

Table 20  Growth of yeast on the waste liquor. 89

Table 21  Composition of the leaf protein samples. 91

Table 22  Composition of the solvent extracted leaf protein samples. 92

Table 23  Papain in vitro digestibility of the leaf protein samples after 24 hours incubation. 94

Table 24  Net protein utilization (NPU) of the rations containing leaf proteins. 95

Table 25  Total amino acids in the leaf protein samples (g % N). 97

Table 26  Available amino acids in the leaf protein samples (g % N). 99
LIST OF FIGURES

Fig. 1 Randomise block design of the experimental plot........... 25

Fig. 2 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 20 days).... 50

Fig. 3 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 30 days).... 51

Fig. 4 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 40 days).... 52

Fig. 5 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 60 days).... 53

Fig. 6 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 80 days).... 54

Fig. 7 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 100 days).... 55

Fig. 8 Effect of different harvesting intervals on the yield/season of crop and its nitrogen content..... 63

Fig. 9 Effect of age on the dry matter & nitrogen content of various cuts and regrowths............. 65

Fig. 10 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (PN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 20 days... 72

Fig. 11 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (PN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 30 days..... 73
Fig. 12 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 40 days.......................... 74

Fig. 13 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 60 days......................... 75

Fig. 14 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 80 days.............................. 76

Fig. 15 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 100 days........ 77

Fig. 16 Effect of different harvesting intervals on the yield season of total nitrogen (TN) in the pulp extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop......................... 80

Fig. 17 Effect of different harvesting intervals on the yield season of fibrous residue (FR) and its nitrogen content................. 86
INTRODUCTION

Proteins are the most important for development of human body and maintenance of good health. Besides contributing to the internal frame work of all cells and to the structure of intracellular substances, protein furnish the amino acids, from which enzymes, hormones, haemoglobin, plasma proteins and many other physiologically active substances are synthesized. Adequate amount of proteins are also essential for normal maintenance of body functions and for growth, maturation, pregnancy, lactation and recovery from injuries and diseases.

Protein deficiency and malnutrition is being experienced by a large population of the world. It has been recently reported that 15 million people in America suffer from sub-nutrition. Over three hundred million children are reported to suffer grossly retarded physical growth and development due to non-availability of sufficient protein and calories (UNESCO report 1979).

The gravity of protein deficiency in Pakistan can be judged from the fact that 150 million kg/year animal proteins are available for 68 million persons. The per capita consumption thus comes to about 6.0 grams per day. This shows that production of protein
in Pakistan is one of the lowest in the world as compared to 75 grams in New Zealand, 61 in Australia, 65 in U.S.A. and Canada, 18 in Japan and 15 in Phillipines (Akram, 1968). This limited supply of protein is leading the population to deterioration of health and physique. The situation seems to aggravate further by the fact that population of Pakistan is increasing very rapidly and is estimated to be 110 million in 1990.

The grave shortage of protein and rapid explosion of population needs immediate attention and calls for firm steps. The approach must be multi-pronged and should include improvement in the conventional as well as exploitation of unconventional sources of proteins. The object should be improvement in the quality as well as quantity of proteins.

Proteins of animal origin which include milk, poultry, eggs, meat, fish are superior in quality as compared to those of vegetable origin. They have complete assortment of essential amino acids, are easily digestible and are of higher biological value. But raising of beef and meat herd is a lengthy process and is most uneconomical.

Slade (1937) reported that direct extraction of protein from grasses was more economical than feeding it to animals who convert it to high grade
protein. Pirie (1942, 1952) also suggested that direct extraction of protein from leaves was more useful. Tender green leaves contain good quality and readily extractable proteins and can be used as a supplement in human diet, provided the fibrous material and other toxic substances are eliminated. However considerable difficulties are involved in the preparation of leaf protein concentrates which are easily digestible, attractive and economical. Attempts have been made and are still in progress at Rothamsted Experimental Station (Davies & Pirie, 1961) and other places (Chayan, 1961), to extract protein from leaves and to use it for edible purposes. Nutritional evaluation of leaf protein is also being extensively investigated so that it can be utilized if a cheap method of production is made available.

Vegetable proteins are available in abundance but they are deficient in some essential amino acids like methionine and lysine and cannot support proper growth and development (Byers et al., 1965). These should either be combined with animal protein or other vegetable proteins which will compliment its amino acid pattern. Large number of workers in different countries have made protein rich food preparations from cotton seed flour (WHO/FAO/UNICEF reports 1959, 1960). Of the special interest are
Incaprina, Allison Flour and Coriib which are rich protein sources (Benar et al., 1959; Alvi et al., 1967). Sur (1961) prepared leaf protein concentrate from fenugreek to supplement protein deficient diet of the South Indians. Guna (1960) extracted proteins from leaves and Kuppuswamy et al. (1958) analysed these protein preparations.

The present studies deal with the cultivation of Persian clover (Trifolium resupinatum) under known conditions of soil and climate. The yield, habitat of the foliage when harvested after different intervals including the regrowth is reported. The changes in nitrogen content of the crop at various stages of growth are also described. The yield of extractable nitrogen, extractable protein nitrogen and other fractions that are obtained on processing the cuts and regrowths of different ages is calculated. Further it shows how the yield of the different fractions varies with the physiological age of plants.

The effects of different methods of drying on the nutritive value of leaf protein concentrates have been also studied. It involves the estimation of in vitro and in vivo digestibilities and the availability of essential amino acids before and after processing. The effects of lipids oxidation products and lipid protein
complex on the enzymatic digestion, availability of amino acids and net protein utilization of the leaf protein concentrates are also discussed.
REVIEW OF LITERATURE

The presence of protein in plants was first demonstrated by Rouelle (1772). Extraction of protein from leaves and its use as an adjunct to human diet was suggested by Erskye (1910) and Pirie (1942, 1952). Protein concentrate from spinach and cabbage leaves was first prepared by Chibnall and Schryver (1920, 1921). Miller and Chibnall (1932) reported that isolation of protein from grasses was more difficult. Chibnall (1933) showed that the major problem in isolation of protein from leaves was the difficulty in breaking the leaf cells. Various methods including plasmolysis of the cell, extraction with ether and mechanical means, were employed to rupture the cell wall. Attempts to extract protein concentrate from leafy materials met limited success due to lower yields and mechanical defects (Tilley et al., 1937).

The separation of protein on laboratory scale by extracting the juice and precipitating the proteinous matter by acidification, organic solvents and precipitating agents like, trichloroacetic acid and phospho-lactic acid was obtained with varying degree of efficiency. Crook (1950) examined the conditions for maximum extraction of protein from tobacco leaves and
outlined the procedure for successful extraction of protein. Crook and Holden (1958) followed this method and extracted the nitrogenous material from leaves of 28 species of plant. Byers (1961) studied the extractability of protein from 60 species grown in Ghana. It was reported that legumes and some weeds were the best sources of easily extractable and good quality protein. Festenstein (1961) discussed the disadvantages of triple roll sugar mill procedure and employed Pirie's disintegrator (1956) for the extraction of nitrogenous matter from leaves of different species. Byers (1965) discussed large scale extraction of protein from leaves of different ages and varieties and concluded that it depended on species, variety, the physiological age and its re-growths. Singh (1961) extracted protein from various Indian plants. Nazir and Shah (1960) studied extractability of proteins from tealty plants that grow abundantly in Pakistan and observed that leguminous and Cruciferous plants were the best sources of easily extractable proteins.

concentrates by spray drying. Akeson et al (1966) prepared leaf protein concentrates from foliage and compared protein production here of the foliage with that of seed and animal crops. Composition of proteins isolated from certain species of Indian flora and the plants growing in Ceylon was also reported, (Valli Davi et al., 1965; Senthilnath and Durand, 1969). Matai et al (1971) estimated total and extractable nitrogen present in the leaves of eleven cultivated and nine wild species of Bengal. Arkell and Festenstein (1971) studied the agronomical factors affecting the yield of extractable proteins. Lu and Kinsella (1972) prepared leaf protein from alfalfa meal processed at 70 to 150°C and reported the composition of extracted proteins. Yasui et al (1973) isolated from 60 to 90% of proteins from clover, radish and spinach leaves by successive treatments with several organic solvents and hot-water or 5% sodium chloride solution. The product isolated was analysed for its amino acid content. De Fremery et al (1974) separated white and green fractions from alfalfa leaves by centrifugation and controlled heating. Retzehart and Kinsella (1973) developed a new procedure for maximum extraction of leaf proteins by using micromill and tribuffer, pH 7.4. The protein extracted from number of species with this
method was tested for its solubility at various pH values.

Pirie (1942, 1952) advocated the large scale production of proteins from leaves to supplement the protein deficient diets. A machinery was developed at Rothamsted Experimental Station for the production of leaf proteins in bulk (Pirie 1953, 1957; Davys & Pirie 1960). Morrison and Pirie (1961) prepared protein on large scale. The product was a mixture of cytoplasmic and chloroplastic proteins and contained 60-70% moisture. Modifications continued but the basic method of extraction remained unaltered. Ahmed et al (1959) described a device based on the steam jet ejector principle for treating large volumes of leaf extract to coagulate protein for use in smaller or industrial scale production of leaf protein. Arckoff and Davys (1971) described a machinery which efficiently extracted leaf proteins of good quality by coagulating the juice with steam, the produced leaf protein cake was rich in essential fatty acids, carotene, vitamin E and minerals. They developed this method for alleviating proteins malnutrition and to allow fodder to be used efficiently in their own country. Knuckles et al (1970, 1972) described different pilot plant extractors for crushing and expression of juice from alfalfa. These authors tested the working efficiency of about six
equipments and reported that twin screw press was superior to other expressions. Spencer et al. (1970, 1971), designed a pilot plant coagulation system capable of handling over 90 gal. of juice/hour. The effects of pH on coagulation of green juice in the pilot plant were also studied. The best starting material for the system was reported to be the juice prepared from ammoniated freshly chopped alfalfa with pH 8.0 - 8.5. Lazar et al. (1971) assembled a pilot plant for continuous separation of protein-xanthophyll coagulum from residue liquor containing alfalfa solubles. A pro-xan process was developed by Booth et al. (1972), Halloran (1972) and Kozmicky et al. (1972) for the production of LPC on commercial scale and the product was used for animal feeding.

The presence of substantial amount of chlorophyll, unsaturated fatty acids and phospholipids in chloroplastic and cytoplasmic protein was reported by earlier workers (Robert, 1953; E. Wysasting, 1937). The formation of lipid-protein complex and adsorption of lipids and their oxidation products on jet in was reported by various workers (Norton and Medman, 1977; Davies and Gill, 1975). Oxidation of unsaturated fatty acids present in leaf protein concentrates was reported by Lee et al. (1964). Some observations
on the oxidative deterioration of lipids of crude proteins were given by Lea and Parr (1961). Garton (1960) observed the presence of 80% unsaturated fatty acids in the lipids extracted from grasses. Mallic et al. (1961) and Debuch (1962) studied the fatty acids of spinach chloroplasts and showed that more than 70% of the acids were unsaturated. Lima et al. (1965) reported that amount of unsaturated fatty acid in leaf protein lipid was 52.1 to 75.1% of the total acids. It was also suggested that rapid oxidation of phospholipids was responsible for the development of unpleasant flavor in leaf protein concentrate.

Shah (1968) studied the changes in leaf protein lipids in vitro and inactivated the enzymes present in LPC by heating it at 100°C in the presence of nitrogen and checked the enzymatic and non-enzymatic deterioration of the proteins by addition of 'amla' (Emblica officinalis) powder.

Singh (1962) observed differences in the nature of nitrogen precipitated by various methods from wheat extract. Singh (1961) studied the changes in leaf sap of several species and reported that the proteolytic enzymes present in leaves converted protein into amino acids at favorable temperature.

Nasir and Shah (1966) incubated Suaeda aegyptiaca leaf juice at 37°C and studied the percentage
breakdown of the protein present in it. Nazir and Shah (1968) noticed changes in the leaf juice of Persian clover (Trifolium resupinatum) caused by the proteolytic enzymes. Myszkoński (1970) investigated the protease activity in wheat leaves during growth and development. The changes in chloroplastic pigments during the preparation of leaf protein was studied by Atkinson and Riddon (1973). It was noticed that carotene and xanthophyll were fairly stable in juice stored for several days at 20°C, but at room temperature these constituents were comparatively less stable. Jee and Gardin (1950) observed a decrease in nutritive value of foodstuffs during heating. This was attributed to the formation of complexes by sugars and amino acids, and destruction of heat labile amino acids. Gieaso et al. (1951) reported that losses in the nutritive value of whole herring meal on heating at 70°C were due to oxidation of lipids. Rees and Hart (1951) found reduction in the amount of available amino acids in heated herring meal. Similar changes were also observed by Miller (1956) in heated fish meal which were reported to be due to the Maillard reaction. According to Lee et al. (1960) the deterioration in the nutritive value of protein was oxidative type during normal storage or on
Wills (1952) reported that oxidative products of unsaturated fatty acids formed during heating were toxic to many enzymes. Buckworth and Wootten (1951) studied the nutritive value of heat protein concentrate as a supplementary source of protein for chicks and rats. It was reported that samples dried at temperature 85°C were highly nutritious whereas those dried at higher temperatures showed a marked decrease in the nutritive value. Carpenet et al. (1962) studied the effects of heat and moisture content on the availability of essential amino acids in fish meal and reported a decrease up to 12% in the samples heated at 85-150°C. Prasada et al. (1962) reported losses in dry weight, moisture, protein and sulphur amino acids when pork protein was heated at 110°C with equal volume of water. Higgin et al. (1965) reported that heat processing decreased the availability of methionine in feeds, thus affecting its total amount. Ellinger & Bagnis (1962), Greenwood & Pearson (1968) and Ellinger & Parker (1969) showed that it could be due to the oxidation of peptide bond methionine to sulphoxide (meto). Pierpoint (1969 a,b) reported that factors responsible for these losses in the availability of -SH groups may arise in the chloroplast material were the presence of quinones.
with thiol groups and the reaction between the 4-quinone and polyphenols with £-amino groups. The effects of various drying processes on the content of crude protein, digestible protein, amino acids, nitrates and carotene in foliage of kidney beans and Alfalfa were studied by Vieleneyer et al. (1969). The losses in true digestibility and enzyme solubility, of proteins on heating were observed by Buchanan (1969). These changes were attributed to the formation of protein complexes with unsaturated fatty acids. Yanez et al. (1970) reported the formation of enzyme resistant bonds between the £-amino group and carboxylic groups from monosaccharides while studying the effects of drying temperatures on the availability of amino acids from fish meal. The mechanism of heat damage in protein was discussed by Thornson and Carpenter (1970). Wilter et al. (1972) compared several methods of drying of heat protein concentrate and evaluated the effects of drying on the product.

Chibnall (1937) carried out amino acid analysis of various protein fractions extracted from the leaves of different species and reported that all the preparations were similar in composition. Hug and Kellor (1948) observed decrease in methionine content with corresponding increase in cysteine with an increase
in leaf age. According to Smith and Yara (1951) and Steward et al. (1951), the composition of leaf protein was influenced by leaf age. The protein extracted from mature leaves contained comparatively less amino acids than protein made from young leaves. Other workers (Bryant & Foster, 1951; Fleshman & Gordon, 1951) reported that amino acid composition of un-fractionated or crude leaf protein from different species was not affected by the physiological age of plants. Cihlar (1956) analyzed some preparations by ion exchange chromatography and confirmed their similarity in amino acid composition. Wilson et al. (1955) reported that amino acid composition of leaf protein isolated from mature and immature was not affected by protein age. Yada et al. (1965), more than thirty preparations from eighteen species and observed that largest differences were in the nine containing amino acids apart from proline and lysine. Vali Bevi et al. (1965) observed that methionine content of protein extracted that eighteen species of leaves grown in the same area at the same time but the amount of lysine was only half or less than that of normally estimated leaves. They then analyzed amino acid analysis of varieties of the protein precipitation made at pH 5.1, which showed that the composition of protein extracted from tropical leaves.
ressembled to that of other leaf protein. The chemical composition of four red clover varieties and their amino acid content in relation to various ages of growth was discussed by Kranz-Drobna (1971). Recently Byers (1971) used the preparations made from the extracts of barley, lupin and other crop plants leaves of various ages and established that cytoplasmic and chloroplastic fractions did not have the same amount of amino acids. As with the water-soluble proteins the composition of these fractions was not influenced by leaf age. Tabanha (1973) analysed about nineteen species of plants grown in Nairobi and Kenya for the protein content and reported that it depended largely on the plant age.

The biological value and digestibilities of some grasses, and protein preparation made from young and mature species were assessed by Davie, et al (1952). Carpenter et al (1952 - 1954) discussed the supplementary value of a by-product from grain processing and also found the value of barley for cattle for feed animals. Effinger (1954) evaluated feed protein concentrates by supplying it in varying amounts. Earle et al (1958) incorporated LPC and fishmeal into the diets of pigs and found no efficiency in providing the proteins of feed utilization between the combined LPC and fishmeal when each provided equivalent amounts of protein. (Calishaw et al.
(1956) estimated nutritive value of animal protein concentrates and studied the effects of processing, addition of cholesterol and oil contents on the nutritive value. Waterlow (1962) studied absorption and retention of amino acids from meat protein by infants recovering from malnutrition. Deacon et al. (1961) observed that the efficiency of utilization of the food containing 5% leaf protein concentrate by pigs was comparable with the diet containing 5% white fish meal. Ford (1962, 1965, 1970) determined microbiologically using Streptococcus zymogenes, the total and available amino acid present in leaf proteins, cytochrome c, chloroplasts, mitochondria and in concentrated leaf proteins. The microflora of leaf protein concentrates and its enzyme digestion with pepsin was observed to increase the amount of available amino acids. Mt. Francis and Kenet (1962) estimated the amount of soluble nitrogen present in various leaf protein concentrates and tried to establish relationship between solubility of the protein and its biological value. Rogers et al. (1962) replenished the amino acid profile of animal leaf proteins in terms to the nutritive value. Chibnall et al. (1961) reported negligible loss in the amino acid after hydrolysis with 70% HCl. Hodgson (1967) determined amino acid composition and nitrogen present in two perennial grasses, Akosom and
and Stakman (1951, 1953) evaluated proteins by having the pepsin permeation test on a mix of proteins. The biological value of leaf protein concentrates was reported higher than wheat, casein, soybeans, and yeast. Miller et al. (1959) gave the practical human diets containing LPC to rats and showed that even without methionine supplementation, the LPC were of good feeding value. The supplementation of wheat flour with LPC was also studied extensively using rats. These estimations of PER and liver nitrogen showed that a mixture in which LPC provided equal amount of protein was superior to a mixture containing other proteins (e.g., unpublished).

Henry and Ford (1955) determined the nutritive value of leaf protein concentrates by means of tests with rats and micro-biological methods. It was reported that biological value of leaf protein concentrate was equal to protein present in legumes, cereal seeds and yeast but true digestibilities were lower due to methionine deficiency. It was also observed that heating significantly decreased the biological value and true digestibility whereas freeze drying and drying over starch in a current of air did not affect the quality of leaf protein concentrate. Smuts (1967) studied nutritive value of ten protein concentrates in rats. Fordyce et al. (1969) studied the effects of
supplementing ragi (Eleusine coracana) diets with lysine or leaf protein on the growth and nitrogen metabolism of children in India. At ordinary level nitrogen intake, retention was equal to rats alone fed at same level. Suba Ran et al. (1969) compared biologically the nutritive value of whole extract, coagulated leaf protein and fractionated chloroplastic & cytoplastic proteins of lucerne. The whole extract was observed unsuitable as food because of association of various undesirable water soluble constituents. Shripalekar et al. (1979) determined the nutritive value of leaf protein from lucerne (Medicago sativa) by the growth responses in rats, at different protein levels and to supplementation with lysine and/or methionine. The nutritive value of unfractinuated leaf protein diets was observed to be improved by the addition of methionine. Helio et al. (1969) discussed the supplementary protein value of leaf protein concentrate. Egusa (1970, b) confirmed the advantages of supplementary methionine in rats diets using Cassava leaf protein concentrates. Low biological value and NPl for un-supplementated LFC were raised to 80 and 77 respectively. Suba Ran and Sing (1969) studied the effect of processing conditions on the nutritive value of lucerne leaf proteins and reported
slower growth in rats fed on acid-coagulated protein as compared to those fed on protein coagulated by heat. Sloc (1976) used growth test in the study of differences between plant species and concluded that differences found were attributable rather to feed intake and digestibility than protein quality. Kinsella (1970) reviewed the current status of plant protein research and outlined the general requirements and benefits of the research concerning food protein.
INFORMATION ABOUT THE PLANT

Persian clover (Trifolium resupinatum) belongs to the family Leguminosae and sub-family Papilionaceae. Locally it is known as "Shatatla".

It requires reasonably fertile heavy soil which has the capacity of retaining water for long periods. It grows well in low lying areas or where the irrigation facilities are available. Cold humid climate is quite suitable for it, but extreme cold is harmful.

Persian clover is cultivated in Western Germany, South America, and Australia. In Egypt and Iran this plant is grown in tracts where the irrigation facilities are available. European countries are cultivating it as an ornamental plant. In North Africa this plant has been given preference over Egyptian Clover (Trifolium alexandrinum) and the area under cultivation is continuously increasing.

Persian clover is cultivated in Northern areas of Pakistan for hay, being cultivated preferably because the plant has great recuperative power and gives high yield in 3-4 successive cuts. The total area in the Punjab, Sind and Baluchistan under the fodder crop is 1,999 acres; in the major portion of which is cultivated Egyptian clover.
(Trifolium alexandrinum) and Persian clover (Trifolium regupilation).
EXPERIMENTAL

a) MATERIALS AND METHODS.

PROCUREMENT OF SEEDS.

The pure seeds of Persian clover (Trifolium resupinatum) were procured by Punjab Agricultural Research Station, Lyallpur.

PREPARATION OF SEED BED.

The land was tilled and smoothed. Both these operations were repeated alternatively five times, finally the land was thoroughly levelled.

RANDOMISE BLOCK DESIGN OF EXPERIMENTAL PlOTS (Comer 1948)

An experimental area about 360 sq.m. (PCSIR Labs.) was divided into eight blocks of equal size (20.1 x 4.6 metre) for having eight replicates. Each block was then sub-divided into six equal parts, that is equal to the number of treatments (20, 30, 40, 60, 80 and 100 days), so that each treatment occurred once and once in each block. The actual position of a particular treatment within the block was chosen at random. The treatments were lettered as D₁, D₂, D₃, D₄, D₅ and D₆. The total number of plots thus selected were 48. The measurement of each plot was 15.41 sq.m. (Fig. 1).
SOIL DESCRIPTIONS.

The soil descriptions were prepared according to soil survey Manual (USDA, 1951) and for the horizons nomenclature followed the soil survey manual supplement (USDA, 1962). Colour notations were according to Munsell (1954) soil colour chart. The outline of series descriptions was according to the FAO (1960) Guideline for soil description and for the soil classification consulted 7th Approximation (1960) and USDA classification system (1967).

SOIL SAMPLING.

About 2-3 kg. of soil was taken from each profile i.e. from the depths of 0-12, 12-20, 20-48, 48-80 and 80-240 cm. The samples were packed separately in polythene lined linen bags, marked and were used for the mechanical and chemical analysis.

TEMPERATURE AND RELATIVE HUMIDITY OF THE ATMOSPHERE.

The maximum and minimum temperatures of a day were read from the Fahrenheit thermometer made by short and Mason London. Relative humidity at the morning and evening was found from "Hair Hygrograph" fabricated by Cassella London Model 1789. From the data thus obtained mean temperature (max. and min.) and mean relative humidity for the month were calculated.
SEED RATE AND METHOD OF SOWING.

50 grams persian clover seeds were sown in each plot of 13.3 sq.m. area, by broadcasting in standing water.

DATE OF SOWING.

Persian clover is sown in Pakistan from middle of September to the end of November. For the present studies it was sown in the second week of October consecutively for three years (1969, 1970 & 1971).

IRRIGATION AND CROP CARE.

The experimental plots were lightly irrigated after 8-10 days of sowing for the young seedlings to establish. Subsequently the plots were irrigated after every 10-15 days subject to the climatic conditions. Weed was removed at regular intervals to facilitate proper growth of the crop.

INTERVAL BETWEEN THE HARVESTS.

The crop was harvested after 20, 30, 40, 60, 80 and 100 days intervals. The regrowths were also cut in the same manner. The first harvest in each case was referred to as 1st cut and the harvests taken after the 1st cut were named as regrowths in the text.
HARVESTING AND WEIGHING.

The plants were cut approximately two inches above the ground level by conventional method i.e. by hand with a sickle. The crop was weighed (kg) immediately after harvesting.

HEIGHT OF PLANTS AND LEAF: STEM RATIO.

About 20 representative plants were taken from each harvest and their height was measured in cm. and the average height was calculated. About 200 g. of leaves were stripped off from the stems and the exact weight of both leaves and stems was found out separately and the leaf: stem ratio was calculated.

EXTRACTION OF PROTEIN.

Protein was extracted from the 20, 30, 40, 60, 80 old and 100 day 1st cuts & regrowths with in 30 minutes after harvesting. The process followed for the extraction of protein was that used by Crook and Holden (1948). Few modifications were made but basic method of extraction remained unaltered.

About 5 kg of foliage was thoroughly washed under running water to eliminate dust and mud sticking to it and to reduce the microbial load. After draining out the water, the foliage was minced in a Crypto-Electric Mincer. The pulp was squeezed through long-cloth by hand pressing for the extraction of juice.
The weight of the pulp was recorded and samples were taken from it for the estimation of dry matter and nitrogen content. The volume of juice was measured and 2 ml samples were taken from it for estimation of extractable nitrogen, proteinous nitrogen and non-proteinous nitrogen. The weight of the fibrous residue (FR) left after the extraction of juice was also taken and samples were taken from it for the estimation of dry matter and nitrogen content.

**SEPARATION OF PROTEIN.**

The juice was heated upto 80 ± 2°C for the co-agulation of the proteins which were separated from the supernatant by gravity filtration using Long-cloth bags. The supernatent left in the proteins was removed by hand pressing. The Leaf protein cake was crumbled with hand and was washed repeatedly with distilled water until the washing was colourless. The washings were discarded.

The crude protein containing lipids, starch, cellulose and minerals was pressed hard with hands to remove the excess of water. The finished product containing about 70% moisture is referred in the text as Leaf Protein Cake (LPC). Its weight was recorded
and samples for the estimation of dry matter and nitrogen content were taken from it. The LPC was stored in a freezer at -20°C till use.

The volume of the supernatant obtained after the heat co-agulation of proteins was measured. Its nitrogen content, proteinous nitrogen and non-proteinous nitrogen, amide nitrogen, sodium, potassium, calcium and iron contents were estimated. The supernatant is mentioned in the text as waste liquor.

**PROPAGATION OF FOOD YEAST ON WASTE LIQUOR:**

**Source of Yeast Culture:**

The culture of *Candida utilis NCYC 197* was obtained from the National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, England and employed for studying growth of the yeast on waste liquor.

**Maintenance of Stock Culture and Preparation of Inocula:**

The yeast strain was maintained on agar medium containing peptone 1.0%; yeast 5.0%; dextrose 0.3%; malt extract 0.3% and agar 2.5%. A broth of the same composition was also prepared and 24 hours old yeast cells were transferred into it aseptically. The cells were allowed to multiply for 24 hours at 30 ± 0.5°C over rotary shaker having 80 RPM. The cell suspension of 0.3 optical density was prepared in distilled water.
at 6000 g employing Beckman Spectrophotometer and
used for inoculation of basal media (waste liquor).
propagation:

50 ml. of the waste liquor, the pH of which
was adjusted to 5.0 was sterilized at 15 p.s.i. for
15 minutes in 250 ml. Erlenmayer flask. 0.5 ml of
the inoculum was aseptically added into it, and the
flasks were kept at rotary shaker at 28 ± 0.5°C having
80 rpm. The pH of the substrate was maintained at
5.0 throughout the incubation period by checking it
after every 12 hours and by adding dilute sodium
hydroxide solution if required.

Harvesting:

The yeast cells were harvested after 48 hours
of incubation by centrifugation. The cells were
suspended in distilled water, centrifuged and dried
at 100°C for 24 hours in an oven. The product was
weighed and its nitrogen content was estimated.

PREPARATION OF LEAF PROTEIN SAMPLES.

Leaf protein cake (LPC) obtained from the 20
day old cut and regrowths was mixed thoroughly and
further processed. Several leaf protein samples were
prepared from the LPC by drying it in an oven at various
temperatures, roller drying, freeze drying and by
extraction with solvents.

**OVEN DRYING.** Four samples of LPC about \(\frac{1}{4}\) kg each were uniformly spreaded (0.6 cm thick layer) over 41.0 x 7.5 x 56.0 cm. size wire gauze trays having four holes in one sq.cm. The trays were placed in the drier. The temperature of the ingoing air was maintained at approximately 70, 80, 90 and 100°C according to which samples were to be dried. Each sample in turn was placed in drier and heated at the fixed temperature until drying was complete, as judged by appearance and touch and was removed. The dried samples were numbered as OD₁, OD₂, OD₃ and OD₄. The oven used for the purpose was Mitchell's drier.

**ROLLER DRYING.** About \(\frac{1}{4}\) kg sample of LPC was dried by passing over steam heated roller, a machine fabricated by Richard Simons and sons Limited. The temperature of rollers varied from 120-135°C during heating on the roller for 15 seconds. The resultant product will be referred as RD.

**FREEZE DRYING.** About \(\frac{1}{4}\) kg of LPC was freeze dried in a Laboratory freeze drier of the type 30 D/35 Edward High Vacuum Ltd., England. The finished product was labelled as FD.

**SOLVENT EXTRACTION.**

(a) Extraction with chloroform; methanol mixture.
About 2 Kg LPC was homogenised in parts with a sufficient quantity of 2:1 (v/v) chloroform:methanol mixture in a Warner blender. After blending for 2-3 minutes the homogenate was filtered through Whatman No.1 filter paper in a Buchner funnel with slight suction. This extraction procedure was repeated until the filtrate was colourless. The solvent was removed from the dehydrated and defatted proteins by keeping it in vacuum oven at 36°C for 24 hours. The dried defatted product was labelled as LPC_a.

(b) Extraction with acetone. About 2 Kg of LPC was homogenised in parts with a sufficient quantity of acetone in a Warner blender for 2-3 minutes. The homogenate was filtered through Whatman No.1 filter paper on Buchner funnel with slight suction. The extraction was repeated until the filtrate was colourless. The solvent treated product was placed in vacuum oven at 36°C for 24 hours for removing the solvent. The finished product will be referred as LPC_b.

**GRINDING OF SAMPLES.**

All the samples of leaf protein were ground so as to pass through the 80 mesh size sieve, packed in polythene bags and stored at room temperature. The machine used for the sample grinding was a Mechanical edge grinder fabricated by Pascall Engineering Co.Ltd., England.
EXTRACTION OF LIPIDS FROM DRIED SAMPLES.

The leaf protein samples oven dried (OD₁, OD₂, OD₃, and OD₄), roller dried (RD) and freeze dried (FD) were divided into three equal parts. First part of each sample was treated with chloroform:methanol (2:1) mixture and second part with acetone, for the extraction of lipids. The third part was left as such. The samples defatted with chloroform:methanol were labelled as OD₁a, OD₂a, OD₃a, OD₄a, RD₁a, and FD₁a respectively, whereas those extracted with acetone were labelled as OD₁b, OD₂b, OD₃b, OD₄b, RD₁b, and FD₁b respectively.

b) STATISTICAL.

"Analysis of variance with two way classification and with several observation per cell" technique was employed for testing the differences between yield of Persian clover when harvested after 20, 30, 40, 50, 60, and 100 days intervals including the regrowths.

For finding out the optimum interval between the successive harvests which could give the maximum yield of Persian clover "least significance difference test" was applied to the mean total yield of crop.

Least significance difference = \( t_{0.5} \times S.E \) (L.S.D).

\[ t_{0.5} = 1.96 \]

\[ S.E. = \sqrt{\text{error mean square}} \]
**ANALYTICAL.**

**MECHANICAL ANALYSIS.**

The mechanical analysis of soil samples was carried out by Hydrometer Method using the calgon solution (Sodium hexa-metaphosphate 50 g/100 ml) as dispersing agent. (Hussain et al, 1968).

**CHEMICAL ANALYSIS.**

For the chemical analysis of soil samples, strictly followed Richard (1954). The flame photometer employed for the estimation of sodium and potassium was of Gallenkamp Model F.W. 500. Electrical conductivity was measured by Soil-Bridge Soil-Tester Reg. USA 9446.

**MOISTURE.**

The moisture content of all the samples was determined by drying in oven at 105°C for 24 hours.

**NITROGEN.**

Nitrogen content of the samples was determined by micro-Kjeldahl method using K₂SO₄;CuSO₄;SeO₂ (9:1:0.02) catalyst.

**PROTEINOUS AND NON-PROTEINOUS NITROGEN.**

2 ml of the juice or waste liquid was treated with an equal volume of 10% Trichloro-acetic acid (TCA) and cooled at + 4°C for 10 minutes. It was centrifuged for
10-15 minutes at 2000 rpm, TCA-insoluble fraction contained the proteinous nitrogen (PN) and the TCA-soluble portion contained non-proteinous nitrogen (NPN). Both the fractions were separated and analysed for the nitrogen content.

**Amide Nitrogen.**

Amide N was estimated by hydrolysis of 2 ml of waste liquor with equal volume of 2N H2SO4 at 100°C for two hours. The ammonia present in the hydrolysate was distilled in a Markham still (Paech and Tracey 1955), collected in 5 ml 2% W/V boric acid and titrated with $\frac{N}{70}$ HCl.

**Estimation of Metal Ions in Waste Liquor.**

Sodium and potassium were estimated photometrically, calcium by Ammonium oxalate method and iron by o-phenanthroline method (Paech and Tracey 1955).

**Determination of pH.**

pH determinations were made with a glass electrode.

**Total Sugars.**

The total sugars in the waste liquor were estimated by Folin and Wu Micro method (A.O.A.C. 1970).
ESTIMATION OF LIPIDS.

The lipids present in the leaf protein samples were determined by two different methods.

Procedure A. 2 g samples of leaf protein were soaked in 30 ml 2:1 (v/v) chloroform:methanol mixture at room temperature \(20 \pm 2^\circ C\) for two hours then filtered through Whatman No. 1 filter paper followed by three 40 ml solvent rinses through the same paper. The lipids extracted were washed with 0.58% Sodium chloride solution using the method of Folch et al. (1957). Chloroform layer containing the lipids was separated and the total lipids present in it were estimated by evaporating a 50 ml of aliquot and taking the weight of residue.

\[
\text{Total lipids} = \frac{\text{wt. of lipids in aliquot} \times \text{vol. of chloroform layer}}{\text{vol. of aliquot}}.
\]

Procedure B. 2 g samples were soaked in 50 ml acetone for two hours at room temperature \(20 \pm 2^\circ C\) then filtered through Whatman No. 1 filter paper. It was followed by three 40 ml solvent rinses through the same paper. The volume of the extract was measured, and a portion of the extract was evaporated to dryness in a tared flask. The weight of lipids residue determined and total lipid in the sample was calculated.
IN VITRO DIGESTIBILITY TEST.

The in vitro hydrolysis of leaf protein samples, by papain was measured as described by Byers (1967a).

IN VIVO TEST.

Net protein utilization (NPU) of all the leaf protein samples was determined by the method of Miller and Bender (1955). Some modifications were made according to Buchanan (1968).

\[ \text{NPU} = \frac{\text{Bn} - (\text{Bk} - \text{Ik})}{\text{In}} \times 100 \]

- \( \text{Bn} \) = mg body N of rats group on leaf protein diet.
- \( \text{Bk} \) = mg body N of rats group on non-protein diet.
- \( \text{In} \) = mg N intake of rats group on leaf protein diet.
- \( \text{Ik} \) = mg N intake of rats group on non-protein diet.

ESTIMATION OF AMINO ACIDS.

The total and available amino acids in the leaf protein samples were estimated by microbiological assay.

CHEMICAL HYDROLYSIS. The acid and alkali hydrolysis of the leaf protein samples for the estimation of total amino acid, was done by the methods described by Ford (1962).

ENZYMIC HYDROLYSIS. Leaf protein samples were hydrolysed with 2% papain for the determination of available amino acids (Ford 1964).
MICRO-ORGANISMS: *Streptococcus zymogenese*, NCDO 592 was used to determine the total and available methionine, tryptophane, leucine, isoleucine, arginine, valine, histidine in hydrolysates. The procedure followed for the assay was as described by Ford (1962). The total and available lysine and phenylalanine was assayed by *Leuconostoc mesenteriodes* ATCC 8042, whereas threonine was estimated with *Streptococcus faecalis* ATCC 8049. The details of procedure are given in Difco suplimentary literature (1968).
RESULTS

Tables 1-3 show the profile of soil and its mechanical and chemical analysis. It is evident from the results that the soil contained an adequate amount of essential nutrients and its texture was suitable for propagation of the crop under investigation.

Table 4 shows the changes in the mean daily temperature and relative humidity from September 1970 to August, 1971. This period covers the season in which Persian clover grows. The fluctuations in maximum and minimum atmospheric temperatures ranged from 19.3 to 41.1°C and from 5.1 to 27.1°C respectively. The variations in relative humidity were from 44 to 90% in the mornings (8.0 A.M.) and 22 to 54% in the evenings (5.0 P.M.).

Tables 5 & 6 show the changes in the height and leaf:stem ratio of the plants when the crop was harvested regularly after 20, 30, 40, 50, 80 and 100 days intervals during the season.

The average height of plants was 16 cm. in the 20 day old 1st cut and it increased to 37 cm. during the successive eight regrowths. However in case of 2nd and 3rd regrowths a slight deviation was observed when the height of plant did not exceed 12 cm. The ratio of stem to leaves gradually increased but the average
## TABLE 1

SOIL DESCRIPTION.

The following profile was described and sampled in a specially prepared pit in the experimental plot of CESLR laboratories, Lajore.

### Profile

<table>
<thead>
<tr>
<th>Layer</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0-12</td>
<td>Brown/dark brown (10YR 4/3) moist and pale brown (10YR 6/2) dry; silty clay loam; massive; very sticky, very plastic, fine moist, very hard dry; few fine and common very fine tubular and interstitial pores; moderately calcareous; few fine roots; clear smooth boundary; pH 8.2.</td>
</tr>
<tr>
<td>A1c</td>
<td>12-30</td>
<td>Brown dark brown (10YR 4/3) moist and pale brown (10YR 6/2) dry; silt loam; massive and very weak coarse subangular blocky; slightly sticky, slightly plastic, friable moist, hard dry; few fine and common very fine tubular pores; moderately calcareous; few pottery pieces; few fine and common very fine roots; clear smooth boundary; pH 8.2.</td>
</tr>
</tbody>
</table>

Cont...
B21  20-48 cm.  Brown/dark brown (10YR 4/3) moist and pale brown (10YR 6/3) dry; common fine and medium distinct olive grey mottles; silty clay; weak coarse subangular blocky; very sticky, very plastic, firm moist, very hard dry; common fine and very fine tubular pores; moderately calcareous (but less than the above horizon); few fine iron-manganese concretions; few fine and very roots; clear smooth boundary; pH 8.2.

B22  48-80 cm.  Brown/dark brown (10YR 4/3) moist and pale brown (10YR 6/3) dry; common fine and medium distinct olive grey mottles; silty clay tending to silty clay loam; weak coarse subangular blocky; very sticky, very plastic, firm moist, very hard dry; common fine and very fine tubular pores; slightly calcareous; very coarse pockets of brown, dark brown (7.5YR 4/4) and moderately calcareous silt loam,

Cont...
containing few fine concretions; fine iron-manganese concretions; few
time and very fine roots; clear wavy boundary; pH 8.2.

(The whole of the horizon was wet).

A23  80-240 cm. Dark greyish brown (10YR 4/2 and 2.5YR
4/2) moist; common fine and medium
distinct yellowish brown mottles
(mottles increase to many after 140 cm)
silty clay; weak coarse subangular
blocky breaking into medium and fine
subangular blocky; very sticky, very
plastic; very firm moist, very hard
dry; common fine and very fine tubular
pores; non-calcareous; common to many
fine and medium iron-manganese concre-
tions; few fine roots; pH 8.2.

Note: The above soil is classified as Typic Cambor-
thid according to 7th Approximation and USDA
system (1967).
### Table 2: Mechanical Analysis of the Soil

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Hydrometer readings</th>
<th>*USDA Sand</th>
<th>**Int. Sand</th>
<th>Clay</th>
<th>*USDA Silt</th>
<th>**Int. Silt</th>
<th>Soil Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncorrected/Corrected</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 sec.</td>
<td>5 min.</td>
<td>2 in.</td>
<td></td>
<td>100-(sand + clay)</td>
<td>100-(sand + clay)</td>
<td></td>
</tr>
<tr>
<td>5120</td>
<td>41.0</td>
<td>26.0</td>
<td>15.0</td>
<td>17.0</td>
<td>100-82 = 18.0</td>
<td>100-52 = 48.0</td>
<td>18.0</td>
</tr>
<tr>
<td>5121</td>
<td>41.0</td>
<td>26.0</td>
<td>15.0</td>
<td>17.0</td>
<td>100-78 = 22.0</td>
<td>100-52 = 48.0</td>
<td>22.0</td>
</tr>
<tr>
<td>5122</td>
<td>41.0</td>
<td>26.0</td>
<td>15.0</td>
<td>17.0</td>
<td>100-80 = 20.0</td>
<td>100-52 = 48.0</td>
<td>20.0</td>
</tr>
<tr>
<td>5123</td>
<td>47.0</td>
<td>11.0</td>
<td>19.0</td>
<td>20.0</td>
<td>90-85 = 15.0</td>
<td>100-56 = 44.0</td>
<td>15.0</td>
</tr>
<tr>
<td>5124</td>
<td>42.0</td>
<td>22.0</td>
<td>9.0</td>
<td>20.0</td>
<td>100-84 = 16.0</td>
<td>100-52 = 48.0</td>
<td>16.0</td>
</tr>
</tbody>
</table>

*United States Department of Agriculture.

**International.
<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th><strong>WSP</strong></th>
<th>(Ca + Mg)</th>
<th>Na</th>
<th>CO₃</th>
<th>HCO₃</th>
<th>Cl</th>
<th>SO₄</th>
<th>Total Cations</th>
<th><strong>Millihoes EC x 10 at 25°C</strong></th>
<th><em><strong>S.A.R.</strong></em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5120</td>
<td>31.0</td>
<td>7.0</td>
<td>9.0</td>
<td></td>
<td>4.5</td>
<td>0.5</td>
<td>0.5</td>
<td>16.6</td>
<td>1.60</td>
<td>4.84</td>
</tr>
<tr>
<td>5121</td>
<td>30.0</td>
<td>3.0</td>
<td>8.0</td>
<td></td>
<td>1.0</td>
<td>0.5</td>
<td>1.2</td>
<td>14.4</td>
<td>1.10</td>
<td>4.56</td>
</tr>
<tr>
<td>5122</td>
<td>37.0</td>
<td>3.0</td>
<td>1.5</td>
<td></td>
<td>0.5</td>
<td>2.0</td>
<td>0.5</td>
<td>15.5</td>
<td>1.50</td>
<td>5.80</td>
</tr>
<tr>
<td>5123</td>
<td>40.5</td>
<td>3.0</td>
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<td>15.5</td>
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<td>2.0</td>
<td>26.0</td>
<td>2.00</td>
<td>8.00</td>
</tr>
</tbody>
</table>

**Water Saturation point.**

**Electrical Conductivity.**

***Sodium Absorption Ratio*** $= \frac{\text{Na}}{\sqrt{\text{Ca} + \text{Mg}}}$. 
TABLE 3.


<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Relative Humidity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>September</td>
<td>35.9</td>
<td>21.0</td>
</tr>
<tr>
<td>October</td>
<td>33.6</td>
<td>17.2</td>
</tr>
<tr>
<td>November</td>
<td>27.9</td>
<td>9.5</td>
</tr>
<tr>
<td>December</td>
<td>21.9</td>
<td>5.7</td>
</tr>
<tr>
<td>January</td>
<td>19.3</td>
<td>5.1</td>
</tr>
<tr>
<td>February</td>
<td>22.4</td>
<td>8.0</td>
</tr>
<tr>
<td>March</td>
<td>27.8</td>
<td>13.1</td>
</tr>
<tr>
<td>April</td>
<td>34.7</td>
<td>18.3</td>
</tr>
<tr>
<td>May</td>
<td>40.2</td>
<td>23.6</td>
</tr>
<tr>
<td>*June</td>
<td>41.1</td>
<td>26.8</td>
</tr>
<tr>
<td>*July</td>
<td>37.0</td>
<td>27.1</td>
</tr>
<tr>
<td>*August</td>
<td>35.8</td>
<td>26.5</td>
</tr>
</tbody>
</table>

*Off Season.
# TABLE - 5.

**Changes in the Height and Leaf: Stem Ratio of Plants in the 20, 30, and 40 Day Old Cuts and Regrowths.**

<table>
<thead>
<tr>
<th>1st Cut and Regrowths</th>
<th><strong>20 DAY</strong></th>
<th><strong>30 DAY</strong></th>
<th><strong>40 DAY</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Height (cm)</td>
<td>Leaf:Stem ratio.</td>
<td>Average Height (cm)</td>
</tr>
<tr>
<td>1st Cut.</td>
<td>16</td>
<td>1:1.1</td>
<td>25</td>
</tr>
<tr>
<td>1st Regrowth.</td>
<td>18</td>
<td>1:1.3</td>
<td>22</td>
</tr>
<tr>
<td>2nd &quot;</td>
<td>11</td>
<td>1:1.2</td>
<td>28</td>
</tr>
<tr>
<td>3rd &quot;</td>
<td>12</td>
<td>1:1.2</td>
<td>33</td>
</tr>
<tr>
<td>4th &quot;</td>
<td>22</td>
<td>1:2.3</td>
<td>50</td>
</tr>
<tr>
<td>5th &quot;</td>
<td>28</td>
<td>1:2.0</td>
<td>70</td>
</tr>
<tr>
<td>6th &quot;</td>
<td>30</td>
<td>1:2.3</td>
<td>-</td>
</tr>
<tr>
<td>7th &quot;</td>
<td>33</td>
<td>1:4.0</td>
<td>-</td>
</tr>
<tr>
<td>8th &quot;</td>
<td>33</td>
<td>1:4.5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Last regrowth taken after 20 days.*
<table>
<thead>
<tr>
<th>1st Cut and Regrowths</th>
<th>60 Day</th>
<th>80 Day</th>
<th>100 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Height (cm)</td>
<td>Leaf:Stem ratio</td>
<td>Average Height (cm)</td>
</tr>
<tr>
<td>1st Cut</td>
<td>57</td>
<td>1:4.0</td>
<td>52</td>
</tr>
<tr>
<td>1st Regrowth</td>
<td>85</td>
<td>1:4.0</td>
<td>100</td>
</tr>
<tr>
<td>2nd &quot;</td>
<td>100</td>
<td>1:9.5</td>
<td>34&quot;</td>
</tr>
</tbody>
</table>

*Last regrowth taken after 20 days.

**Last regrowth taken after 80 days.
remained between 1:1 to 1:4.5. The average height of plants measured in 30 day old 1st cut was 25 cm. which increased up to 70 cm. in the subsequent six regrowths. The leaf:stem ratio ranged from 1:2.3 to 1:5.3. The plants attained the height of about 27 cm. old in case of 40 day 1st cut and later on gradually increased to 97 cm. from the 1st to 3rd regrowth. The last regrowth could grow up to 38 cm. only because the season was over. The leaf:stem ratio varied from 1:2.5 to 1:7.0 (Table 5). The height of plants in the 1st cut of 60 and 80 day was 47 and 52 cm. respectively which increased to 85 cm. in the 60 day old 1st regrowth. The height of plants was about 100 cm. in the 2nd regrowth of 60 day, and 1st regrowth of 80 day. The 2nd regrowth of 80 day was only 20 days old and the height of plants remained 34 cm. The leaf:stem ratio in the 1st cut and regrowths of 60 & 80 day varied from 1:3.5 to 1:9.8. The height of the plants in the 100 day old 1st cut was 85 cm. which increased to the maximum (105 cm.) in the regrowth and leaf:stem ratio range from 1:4.0 to 1:24.0 (Table 5).

Figs. 2-7 show changes in the yield of fresh and dry weights and nitrogen content of 1st cut and regrowths when harvested after 20, 30, 50, 60, 80 and 100 days intervals during the season.
FIG. 2 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 20 days).
FIG. 3

Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 30 days).

Fresh weight = ○
Dry weight = △
Nitrogen content = ▽

---

Nitrogen content

---

Ton / Hectare

---

Dry weight

---

Ton / Hectare

---

Fresh weight

---

2.6

5.2

7.8

10.4

15.6

18.2

20.8

23.4

0.55

1.0

1.95

2.20

2.75

3.30

3.85

4.40

5.05
Fresh weight = ●
Dry weight = ○
Nitrogen content = △

FIG. 4 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 40 days).
FIG. 5
Changes in the nitrogen content of the crop (harvesting interval 60 days).

Nitrogen content vs. Fresh weight.

1st cut: Regrowth dry weights.

Dry weight = 0
Nitrogen content = A

Fresh weight vs. Dry weight vs. Tons/hec.

Table:

<table>
<thead>
<tr>
<th>Fresh weight</th>
<th>Tons/hec.</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>0.06</td>
<td>1.0</td>
</tr>
<tr>
<td>0.08</td>
<td>0.12</td>
<td>2.0</td>
</tr>
<tr>
<td>0.12</td>
<td>0.18</td>
<td>3.0</td>
</tr>
<tr>
<td>0.16</td>
<td>0.24</td>
<td>4.0</td>
</tr>
</tbody>
</table>
FIG 7 Changes in the fresh & dry weights and nitrogen content of the crop (harvesting interval 100 days).

Fresh weight

Dry weight

Nitrogen content

Nitrogen content = 0.05

Fresh weight = 0.10

Dry weight = 0.15

Ton / Hactare

Ton / Hactare
The harvesting of crop after regular intervals of 20 days gave nine cuttings in a season. The fresh and dry weights and nitrogen content of the 1st cut and regrowth varied from 3.85 to 15.71, 0.39 to 1.81 and 0.03 to 0.08 Ton/hectare respectively. The 5th regrowth gave comparatively more yield in terms of fresh and dry weights and nitrogen content among the nine cuttings. The increase in these constituents was approximately three folds as compared to the 1st cut (Fig.3). Six cuttings were obtained when the interval between the harvests was 30 days. The variations in fresh and dry weights and nitrogen content were from 9.11 to 21.38, 1.17 to 3.57 and 0.05 to 0.17 Ton/hectare respectively. The 3rd regrowth showed better yield and nitrogen content than the other harvests. The fresh and dry weights as well as the nitrogen content were twice as compared to the 1st cut. (Fig.3). An increase in the harvesting intervals up to 60 days gave four regular cuttings and one supplementary cut of 20 days old which was counted as 4th regrowth. The fresh and dry weights and nitrogen content ranged between 12.10 to 25.49, 1.47 to 2.90 and 0.06 to 0.11 Ton/hectare respectively. There was noticed a two fold increase in the fresh and dry weights and nitrogen content of the 2nd regrowth (Fig.4.). Harvesting the crop after 60 and 80 days intervals reduced the number of cuttings to three. The last regrowth in case of 80 day old harvests was of 20 days and was considered as
2nd regrowth. The fresh and dry weights and nitrogen content of the 60 & 80 day old 1st cut and regrowths varied from 4.67 to 11.10, 1.35 to 4.65 and 0.038 to 4.48 Ton/haeatre respectively. Sixty day old 1st regrowth gave the maximum fresh weight yield 71.09 Ton/haeatre whereas 80 day old 1st regrowth showed the maximum yield of dry weight and nitrogen content i.e., 4.65 and 4.48 Ton/haeatre respectively (Fig. 5 & 6). Harvesting after an interval of 100 days gave only two cuttings in a season. The 1st regrowth was taken after 80 days instead of 100 days because the season was over. The fresh and dry weights and nitrogen content of 1st cut were 25.66, 2.81 and 0.092 Ton/haeatre respectively. These values decreased to 4.11, 2.39 and 0.042 Ton/haeatre respectively in the 1st regrowth (Fig. 7).

Table 8 shows the analysis of variance of the data given in table 7. The statistical evaluation showed highly significant variations (1% level) in the yield of crop when harvested after an interval of 20, 30, 40, 60, 80 and 100 days, including the regrowths.

Table 10 shows the results of “least significance difference test” applied to the mean total yield of the crop presented in the Table 9. The test showed insignificant difference between the mean total yields of crop, when it was harvested after an interval of 20, 30 and 40
## FRESH WEIGHT YIELD (kg/ha) OF PERSIAN CLOVER FROM EACH

**AFTER AN INTERVAL OF 20, 30, 40, 60,**

<table>
<thead>
<tr>
<th>Block No</th>
<th>First Cut</th>
<th>20 DAY</th>
<th>Regrowth</th>
<th>30 DAY</th>
<th>Regrowth</th>
<th>1st Cut</th>
<th>20 DAY</th>
<th>Regrowth</th>
<th>30 DAY</th>
<th>Regrowth</th>
<th>1st Cut</th>
<th>20 DAY</th>
<th>Regrowth</th>
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<th>20 DAY</th>
<th>Regrowth</th>
<th>3rd Cut</th>
<th>20 DAY</th>
<th>Regrowth</th>
<th>4th Cut</th>
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<th>Regrowth</th>
<th>5th Cut</th>
<th>20 DAY</th>
<th>Regrowth</th>
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<tr>
<td>1</td>
<td>2000.0</td>
<td>948.8</td>
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<td>420.8</td>
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<td>70.00</td>
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<td>84.8</td>
<td>15462.0</td>
<td>880.8</td>
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<td>17127.2</td>
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<td>12742.0</td>
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<td>420.8</td>
<td>70.00</td>
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<td>84.8</td>
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<td>880.8</td>
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<td>940.8</td>
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<td>70.00</td>
<td>19220.0</td>
<td>1816.8</td>
<td>84.8</td>
<td>15512.0</td>
<td>880.8</td>
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</tbody>
</table>
## TABLE 8

### ANALYSIS OF VALENCE OF DATA FROM TABLE 7

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</thead>
<tbody>
<tr>
<td>DAYS</td>
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<td>25599.89</td>
<td>4119.98</td>
<td>8.153</td>
<td>3.02</td>
<td>Sig.</td>
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<td>Blocks</td>
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<td>7480.02</td>
<td>1069.29</td>
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<td>Int.</td>
<td>35</td>
<td>2131.01</td>
<td>60.97</td>
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<tr>
<td>Error</td>
<td>176</td>
<td>110520.96</td>
<td>627.96</td>
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<td>Total</td>
<td>223</td>
<td>140634.64</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

S.O.V. = Source of Variation.  
D.F. = Degrees of Freedom.  
S.S. = Sum of Squares.  
M.S. = Mean of Squares.  
F. = Calculated value of F-ratio.  
Inf. = Tabulated value of F-ratio.
TABLE 9.

MEAN TOTAL YIELDS OF THE CROP WHEN HARVESTED AFTER 20 TO 100 DAYS INTERVALS INCLUDING THE REGROWTHS.

\[ \begin{align*}
D_{20}^- &= 80287.35 \\
D_{40}^- &= 89620.80 \\
D_{80}^- &= 43978.40 \\
D_{100}^- &= 59077.05
\end{align*} \]

\[ \begin{align*}
D_{20}^+ &= 80785.85 \\
D_{40}^+ &= 52354.65 \\
D_{80}^+ &= 59077.05
\end{align*} \]
### Table 20

**Statistical Analysis of the Data Given in Table 5.**

<table>
<thead>
<tr>
<th>Mean Difference</th>
<th>Value</th>
<th>L.S.D.</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{10} - D_{20}$</td>
<td>0.0988,50</td>
<td>1.358,557</td>
<td>Insig.</td>
</tr>
<tr>
<td>$D_{30} - D_{40}$</td>
<td>0.908,05</td>
<td></td>
<td>Insig.</td>
</tr>
<tr>
<td>$D_{30} - D_{60}$</td>
<td>1.937,20</td>
<td></td>
<td>Sig.</td>
</tr>
<tr>
<td>$D_{10} - D_{80}$</td>
<td>0.9078,00</td>
<td></td>
<td>Sig.</td>
</tr>
<tr>
<td>$D_{10} - D_{100}$</td>
<td>0.908,80</td>
<td></td>
<td>Sig.</td>
</tr>
</tbody>
</table>

*Least significance difference.*
days. But the harvests taken after 60, 80 and 100 day intervals showed significant difference among the yields.

Fig. 8 compares the total yields of fresh and dry weights and nitrogen content of the crop when it was harvested regularly after an interval of 20, 30, 40, 60, 80 and 100 days for a period of six months.

When the intervals between the successive cuttings were kept 20, 30 and 40 days, the total yield in terms of fresh weight was 80.79, 80.59 and 80.67 where as the dry weight was 10.21, 11.23 and 10.75 Ton/ha/acre/season respectively. The total nitrogen content decreased with an increase in the age of crop. Maximum total nitrogen content (0.458 Ton/ha/acre/season) was observed in the 20 day old harvests. It decreased to 0.423 and 0.380 Ton/ha/acre/season in the 30 and 40 day old harvests respectively. Any how harvesting after an interval of 30 days gave better yield of the crop than 20 and 40 day old cuts and regrowths. The yield was about 7.5% more in terms of fresh weight and 3.5 to 6% more on the dry weight basis. An increase in an interval between the successive regrowths from 60 to 100 days resulted in a marked decrease in the total yield of fresh and dry weights as well as total nitrogen content. The gradual decrease noticed in these constituents in the 60, 80 and
Fig. 8 Effect of different harvesting intervals on the yield/season of crop and its nitrogen content.
100 day old 1st cuts and regrowths was from 52.35 to 29.68, 8.70 to 5.10 and 0.275 to 0.135 ton-moisture/season respectively.

Fig. 9 shows changes in percentage of dry matter and nitrogen content of the crop harvested after 20 - 100 days intervals during the season.

The dry matter varied from 9.31 to 13.0% and nitrogen content from 3.06 to 5.0% in the 20 day old first seven cuttings, 30 day old first four cuttings, 40 day old first three cuttings, 60 day old first two cuttings (1st cut and regrowths) and in the 1st cuts of 80 and 100 day old crop. Later on dry matter increased from 13.0 to 17.05% and nitrogen content decreased from 5.0 to 4.87% in the 7th & 8th regrowths of 20 day, 4th & 5th regrowths of 30 day, 3rd & 6th regrowths of 40 day, 2nd regrowth of 60 day, 1st & 2nd regrowths of 80 day and last regrowth of 100 day.

Table 11 & 12 show changes in percentage of total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of 20, 30, 40, 60, 80 and 100 day 1st cuts and regrowths.

The pattern of TN, EN, and PN was observed irregular. Twenty day old 1st cut contained 3.10% TN, 53.80% of this was the EN and 46.2% was the PN (ICA insoluble). Although the amount of TN increased by 12-19%
FIG. 9 Effect of age on the dry matter & nitrogen content of various cuts and regrowths.
### Table 1:

Changes in Total Nitrogen (TN) in the Dry Matter of Pulp, Extractable Nitrogen (EN) and Proteinous Nitrogen (PN) in the Juice of the Crop Harvested After an Interval of 20 to 40 Days.

<table>
<thead>
<tr>
<th>Type of Harvest</th>
<th>20 Day</th>
<th>30 Day</th>
<th>40 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN %</td>
<td>EN %</td>
<td>TCA Insoluble</td>
</tr>
<tr>
<td>1st Cut</td>
<td>4.73</td>
<td>52.80</td>
<td>45.14</td>
</tr>
<tr>
<td>1st Regrowth</td>
<td>4.51</td>
<td>52.93</td>
<td>46.59</td>
</tr>
<tr>
<td>2nd</td>
<td>4.90</td>
<td>46.51</td>
<td>46.01</td>
</tr>
<tr>
<td>3rd</td>
<td>4.00</td>
<td>47.22</td>
<td>39.32</td>
</tr>
<tr>
<td>4th</td>
<td>4.01</td>
<td>40.62</td>
<td>38.10</td>
</tr>
<tr>
<td>5th</td>
<td>4.24</td>
<td>51.71</td>
<td>52.04</td>
</tr>
<tr>
<td>6th</td>
<td>4.45</td>
<td>53.80</td>
<td>42.48</td>
</tr>
<tr>
<td>7th</td>
<td>3.80</td>
<td>50.75</td>
<td>37.82</td>
</tr>
<tr>
<td>8th</td>
<td>3.10</td>
<td>38.04</td>
<td>23.84</td>
</tr>
</tbody>
</table>

*Regrowth harvested after 20 days, as the season was over.*
### TABLE 12

**Changes in total nitrogen (TN) in the dry matter of pulp, extractable nitrogen (EN) and proteinous nitrogen (PN) in the juice of the crop harvested after an interval of 60 to 100 days.**

<table>
<thead>
<tr>
<th>Type of Harvest</th>
<th>60 Day</th>
<th>80 Day</th>
<th>100 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN %</td>
<td>EN %</td>
<td>PN %</td>
</tr>
<tr>
<td>1st Cut.</td>
<td>3.55</td>
<td>51.61</td>
<td>84.31</td>
</tr>
<tr>
<td>1st Regrowth.</td>
<td>3.45</td>
<td>50.77</td>
<td>84.07</td>
</tr>
<tr>
<td>2nd</td>
<td>2.66</td>
<td>27.11</td>
<td>79.88</td>
</tr>
</tbody>
</table>

*Regrowth harvested after 20 days as the season was over.*
in the pulp of first four regrowths, but the amount of EN and PN in the juice decreased from 53.80 to 46.02% and 45.24 to 38.10% respectively. It was followed by an increase in EN and PN up to 53.80% and 42.48% in the 5th & 6th regrowths respectively. A sharp decrease was observed in the TN, EN, and PN of 7th & 8th regrowths. The decrease in the TN was up to 1.10% and in the EN and PN was up to 38.04 and 23.41% respectively. This decrease was accompanied by an increase in the fibre content.

The percentage of TN in the 30 day old 1st cut and regrowths varied from 5.20 to 2.50%. The amount of EN and PN in the 1st cut and 1st regrowth was between 51.36 to 51.74% and 42.82 to 41.18% respectively. These values decreased from 51.74 to 44.88% and 44.18 to 17.88% respectively in the 2-5th regrowths in an irregular manner.

40 day old harvests also showed irregular pattern. The decrease in TN was from 4.6 to 2.5% in the 1st cut and the subsequent regrowths. Where as the relative decrease in EN and PN was from 52.16 to 40.79% and 42.38 to 19.66% respectively.

A continuous decrease from 3.55 to 2.0% was noticed in TN of the 60, 80 and 100 day old 1st cuts and in their respective regrowths (Table 12). The amount of EN and PN
also showed a similar trend. The decrease in EN and PN was from 51.6% to 27.11% and 41.31 to 7.88% respectively in the 60 day old 1st cut and regrowths, 49.09 to 41.38% and 32.30 to 25.16% respectively in 80 day old 1st cut and regrowths, 42.67 to 25.00% and 25.67 to 1.50% in the 100 day old 1st cut and regrowth respectively.

The percentage of protein nitrogen (PN) precipitated by heating the juice to 80 ± 2°C was about 5-18% lesser than PN precipitated by TCA in 30-100 day old 1st cuts and their respective regrowths. However the pattern was similar to that of TCA precipitable PN (Table 11 & 12).

Table 13 shows the changes in non-proteinous nitrogen (NPN) as percentage of the total nitrogen in the juice obtained from 20, 30, 40, 60, 80 and 100 day old cuts and regrowths. The NPN in the juice increased from 16.3 to 37.8% with an increase in the age of cuts from 20 to 100 days. The juice extracted from eight regrowths of 20 day old crop showed steady increase in NPN from 14.2 to 40.7% except the 2nd regrowth which contained about 10% lesser NPN than the 1st regrowth. The increase in NPN in the juice extracted from five regrowths of 30 day old crop was from 17.1 to 60.7% and in 10 day old four regrowths was from 8.8 to 56.4%.
<table>
<thead>
<tr>
<th>Harvesting intervals (days)</th>
<th>1st Cut.</th>
<th>Regrowths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>20</td>
<td>16.2</td>
<td>15.5</td>
</tr>
<tr>
<td>30</td>
<td>17.0</td>
<td>14.1</td>
</tr>
<tr>
<td>40</td>
<td>18.8</td>
<td>21.9</td>
</tr>
<tr>
<td>60</td>
<td>20.0</td>
<td>26.7</td>
</tr>
<tr>
<td>80</td>
<td>34.4</td>
<td>43.0</td>
</tr>
<tr>
<td>100</td>
<td>37.8</td>
<td>94.0</td>
</tr>
</tbody>
</table>

*Regrowths harvested after 20 days, as the season was over.

**Regrowths harvested after 80 days as the season was over.
The NPN estimated in the juice of 60 and 80 day old regrowths varied from 26.7 to 65.10%. The juice extracted from the last regrowth of 80 day contained 44% lesser NPN than the juice obtained from 60 day old last regrowth, because it was harvested after 20 days. The maximum NPN (94.0%) was noticed in the juice prepared from the 100 day old last regrowth.

Fig. (10-15) show the yield (kg/hectare) of TN, EN and PN (heat precipitable) in the 20,30,40,60 80 and 100 day old cuts and regrowths.

The yield of TN in the 20 day old 1st cut and regrowths was between 28.1 to 80.1 kg/hectare. Whereas the amount of EN and PN in these harvests ranged from 13.4 to 43.0 and 8.7 to 32.1 kg/hectare respectively. The 5th regrowth contained more TN, EN and PN than the other harvests. The increase in TN and EN was approximately 2.5 times but in the PN was about three fold as compared to 1st cut. (Fig. 10).

The leaf protein cake (LPC) prepared from the 1st cut and first seven regrowths of 20 day old crop contained 56.4 to 63.0% protein and was of bright green colour. But the concentrate obtained from the 8th regrowth contained 51.0% proteins and was brownish green.
Fig. 10 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 20 days.
Fig. 11 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 30 days.
Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 40 days.
Fig. 13 Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 60 days.
Fig. 14. Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 80 days.
Fig. 15  Changes in total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop harvested after an interval of 100 days.
30 day old 1st cut and regrowths contained more TN as compared to the 20 day old harvests and it was estimated between 45.6 to 101.2 Kg/haectare. The yield of EN and PN was also more and ranged between 24.8 to 54.3 and 9.5 to 35.0 Kg/haectare respectively. The amount of TN, EN and PN was almost double in the 3rd regrowth as compared to the 1st cut. (Fig. 11).

The LPC prepared from 30 day old 1st cut and the first three regrowths contained 54.0 to 68.0% proteins, whereas that from the last regrowth contained 48.0% proteins.

The quantity of TN in the 40 day old 1st cut and the subsequent four regrowths further increased from 54.3 to 106.1 Kg/haectare which was also accompanied by an increase in EN and PN. The variations in the former were from 19.4 to 35.1 and the latter were from 9.5 to 37.4 Kg/haectare respectively. The 2nd regrowth was the best among these 40 day old harvests, it yielded 77% and 68% more EN and PN respectively than the 1st cut. (Fig. 12).

The protein content of LPC prepared from the 40 day old 1st cut and the first two regrowths varied from 54.0 to 57.6%. It was 48.0% in the LPC of the last regrowth.

Total nitrogen (TN) content of 60 day old 1st cut and the regrowths varied from 63.8 to 110.9 Kg/haectare.
The amount of EN and PN was between 23.1 to 56.8 and 6.2 to 42.0 kg/hectare respectively. The 2nd regrowth showed the maximum yield of EN, i.e. 42.0 kg/hectare (Fig. 13). The amount of TN in the 80 day old three harvests (1st cut and subsequent two regrowths) ranged from 37.5 to 147.8 kg/hectare. The yield of EN and PN in these harvests varied from 15.5 to 69.8 and 9.0 to 39.8 kg/hectare respectively. The maximum amount of EN (69.8 kg/hectare) was noticed in the 2nd regrowth of 80 day old crop (Fig. 14). The quantities of TN, EN, and PN in the 100 day old 1st cut were 91.4, 39.0 and 21.9 kg/hectare respectively which decreased to 44.1, 10.3 and 0.58 kg/hectare in the last regrowth (Fig. 15).

The I.DC prepared from 60 and 80 and 100 day old 1st cuts and the 1st regrowths of 60 and 80 day contained 54.6 to 58.8% proteins and was dark green in colour. Whereas the product isolated from their last regrowths had 44.0 to 48.0% proteins and was brownish green.

Fig. 16 shows separately the total yield (Kg/hectare/season) of TN, EN and PN in the 20, 30, 40, 60 and 100 day old cuts and regrowths.

20 day old nine harvests (1st cuts and eight regrowths) yield maximum TN, EN, and PN, i.e. 428.4, 211.7
Fig 1.5 Effect of different harvesting intervals on the yield/season of total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of crop.
and 150.2 Kg/hactare/season. An increase in the age of the cuts and the regrowths from 20-100 days showed a regular decrease in the yield of TN, from 428.1 to 132.3 Kg/hactare/season. It was also accompanied by a decrease in the yield of EN from 211.7 to 59.3 and PN from 150.2 to 22.5 Kg/hactare/season.

Table 14 indicates total yield (Kg/hactare/season) of LPC (D.M. basis) obtained from 20, 30, 40, 60, 80 and 100 day old cuts and regrowths and the amount of protein present in it. A progressive decrease from 1507.4 to 246.3 Kg/hactare/season was observed in LPC when age of the harvests increased from 20-100 days. The amount of protein in LPC also decreased from 901.2 to 135.2 Kg/hactare/season. Twenty day old harvests gave maximum yield of LPC and protein i.e. 1507 and 901.2 Kg/hactare/season respectively.

Table 15 & 16 show the changes in percentage of fibrous residue (FR) left after extraction of juice from 20, 30, 40, 60, 80 and 100 day old cuts and regrowths and its N content. The percentage of FR increased and its N content decreased with the maturity of crop. The increase in FR was from 53.07 to 60.77% in the 20 day old 1st cut and the subsequent regrowths. The nitrogen content of the FR varied from 4.28 to 2.35%. The FR increased to 62.19% and to 71.59% in the 30 and
# Table 14

**Effect of Different Harvesting Intervals on the Total Yield of Leaf Protein Care (LPC) and Its Protein Content.**

<table>
<thead>
<tr>
<th>No. of harvests, season</th>
<th>Harvesting intervals, (days)</th>
<th>Yield of LPC Kg/haeacre/season</th>
<th>Protein content (N x 6) Kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>20</td>
<td>1507.50</td>
<td>901.2</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>1443.11</td>
<td>810.0</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>1319.50</td>
<td>715.2</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>761.50</td>
<td>436.0</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>769.90</td>
<td>431.2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>246.30</td>
<td>153.2</td>
</tr>
<tr>
<td>Type of harvest</td>
<td>20 DAY</td>
<td>30 DAY</td>
<td>40 DAY</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>FR %</td>
<td>N content % in dry matter</td>
<td>FR %</td>
</tr>
<tr>
<td>1st Cut.</td>
<td>53.07</td>
<td>3.36</td>
<td>56.7</td>
</tr>
<tr>
<td>1st regrowth.</td>
<td>54.4</td>
<td>3.83</td>
<td>54.03</td>
</tr>
<tr>
<td>2nd</td>
<td>54.90</td>
<td>4.28</td>
<td>58.54</td>
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<tr>
<td>3rd</td>
<td>54.01</td>
<td>4.10</td>
<td>53.30</td>
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<tr>
<td>4th</td>
<td>53.79</td>
<td>3.94</td>
<td>54.31</td>
</tr>
<tr>
<td>5th</td>
<td>54.54</td>
<td>3.50</td>
<td>62.19</td>
</tr>
<tr>
<td>6th</td>
<td>53.07</td>
<td>3.66</td>
<td>-</td>
</tr>
<tr>
<td>7th</td>
<td>58.34</td>
<td>3.15</td>
<td>-</td>
</tr>
<tr>
<td>8th</td>
<td>60.77</td>
<td>2.35</td>
<td>-</td>
</tr>
</tbody>
</table>

*Regrowth harvested after 20 days as the season was over.
<table>
<thead>
<tr>
<th>Type of harvest</th>
<th>60 Day</th>
<th>80 Day</th>
<th>100 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FR %</td>
<td>N content % in dry matter</td>
<td>FR %</td>
</tr>
<tr>
<td>1st cut</td>
<td>54.60</td>
<td>2.91</td>
<td>62.75</td>
</tr>
<tr>
<td>1st regrowth</td>
<td>54.39</td>
<td>3.05</td>
<td>66.74</td>
</tr>
<tr>
<td>2nd</td>
<td>83.48</td>
<td>2.30</td>
<td>82.86</td>
</tr>
</tbody>
</table>

*Regrowth taken after 20 days as the season was over.*
10 day old 1st cuts and regrowths respectively. This was accompanied by a change in the nitrogen content from 3.28 to 2.06% (Table 1).

The FR in the 50 day old 1st cut and 1st regrowth was 51.5 and 52.6%, respectively. It increased to 83.48% in the 3rd regrowth which was accompanied by a decrease in its nitrogen content from 3.05 to 2.30%. The percentage of FR did not exceed 66.79% in the 80 day old harvests. The nitrogen content varied from 2.85 to 2.1%. The harvests were obtained when the interval between the harvests was 100 days. The FR of the 1st cut was 12%, whereas that of the subsequent regrowths was 87.3%. The nitrogen content of the FR of these 2 harvests was 2.83% and 1.6% respectively (Table 1).

Fig. 1, shows the changes in the total yield (Kg/hactare/season) of FR and the amount of unextractable nitrogen in the 20-100 day old cuts and regrowths. The yield of FR in the 10 day old harvests was 3770.7 Kg/hactare/season. This increased to maximum i.e., 6412.5 Kg/hactare/season in the 10 day old harvests. The amount of unextractable nitrogen was 190.2 and 199.2 Kg/hactare/season in the 20 and 30 day old harvests respectively.

The total yield of FR and the amount of unextractable nitrogen in FR gradually decreased from 6162.8 to 3991 and 170.9 to 79.6 Kg/hactare season, when the age
Fig. 17 Effect of different harvesting intervals on the yield/season of fibrous residue (FR) and its nitrogen content.
of crop increased from 30 - 100 days.

Table 17 shows the changes in the nitrogen content of the waste liquor and its volume (litre/ha/year/season) in the 20 - 100 day old cuts and regrowth. The volume of waste liquor increased from 67.3% to 67.5% litre/ha/year/season in the 20 - 100 day old cuts and regrowth. It was followed by a continuous decrease from 67.3% to 67.0% litre/ha/year/season with an increase in the age of crop from 30 - 100 days. The amount of nitrogen that could not be precipitated from the liquor by heating at 80 ± 5°C and appeared in the waste liquor varied from 33.85 to 35.27 kg/ha/year/season in the 20 - 80 day old cuts and regrowth. The amount of nitrogen decreased to 35.87 kg/ha/year/season in the waste liquor of 100 day old harvests. However, the percentage of nitrogen content in the waste liquor increased from 30.9% to 32.5% with an increase in the age of the crop from 20 - 100 days.

Table 18 & 19 show chemical analysis of the waste liquor. It is evident from the results that waste liquor contained sufficient amount of mineral, sugars and nitrogen, and it could be used for preparation of yeast (Candida utilis).

Table 20 shows the yield of yeast (Candida utilis) when propagated on the waste liquor. It was 5.3 litre of the waste liquor or 1.1 M. liters. The total
<table>
<thead>
<tr>
<th>Harvesting Intervals</th>
<th>No. of Harvest Season</th>
<th>Total Volume of Waste Liquor, Litre/ha/season</th>
<th>In-copurifiable Nitrogen Content, kg/ha/season</th>
<th>Nitrogen Content, 1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9</td>
<td>64525</td>
<td>50.24</td>
<td>0.086</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>56120</td>
<td>76.94</td>
<td>0.087</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>52738</td>
<td>71.15</td>
<td>0.084</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>51428</td>
<td>63.82</td>
<td>0.088</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>47135</td>
<td>56.80</td>
<td>0.123</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>19803</td>
<td>25.87</td>
<td>0.195</td>
</tr>
</tbody>
</table>
### Table 18.

**Composition of Waste Liquor** *(g/100 ml)*

<table>
<thead>
<tr>
<th>Dry weight</th>
<th>Total sugars</th>
<th>Nitrogen content</th>
<th>Amid Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.13</td>
<td>0.89</td>
<td>0.082</td>
<td>0.019</td>
</tr>
</tbody>
</table>

### Table 19.

**Mineral Composition of the Waste Liquor** *(mg/litre)*

<table>
<thead>
<tr>
<th>Calcium</th>
<th>Iron</th>
<th>Sodium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>108.30</td>
<td>0.41</td>
<td>1745.30</td>
<td>1895.30</td>
</tr>
</tbody>
</table>

### Table 20.

**Growth of Yeast on the Waste Liquor**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Yield (g/litre)</th>
<th>Yield kg/batch, season</th>
<th>Protein content (N x 6) Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida utilis</td>
<td>5.3</td>
<td>312.38</td>
<td>178.4</td>
</tr>
</tbody>
</table>

*Waste liquor from 20-40 day old cuts and regrowths, taken in a season.*
yield of yeast (Kg/hactare/season) calculated was 350 Kg., which on protein basis comes to be 178.4 Kg/hactare/season.

Table 21 shows the composition of several leaf protein samples. The moisture content of LPC was 73.53%. The other samples FD, OD$_4$ to OD$_7$ and RD contained from 5.09 to 6.51% moisture. The protein content of these samples on dry matter basis was about 60%. The chloroform:methanol (2:1) mixture extracted 27.88% and 27.19% lipids from LPC and FD respectively. The amount of lipids extracted from the heated samples (OD$_1$ to OD$_4$ and RD) decreased from 27.88 to 24.01%. The lipids extracted with acetone also showed the same trend. However the amount extracted with acetone was approximately 3 - 11% lesser than that extracted with chloroform:methanol mixture.

Table 22 shows the moisture and protein contents of the samples extracted with chloroform:methanol mixture or acetone. The protein content of chloroform:methanol treated samples (LPC$_a$, FD$_a$, OD$_{1a}$ to OD$_{4a}$, and RD$_a$) was between 75.0 to 83.7% on dry matter basis. The samples FD$_b$, extracted with acetone (LPC$_b$, OD$_{1b}$ to OD$_{4b}$, and RD$_b$) had comparatively less protein i.e. from 71.28 to 81.24%. That was due to the small amount of lipids left in the material after acetone extraction.
TABLE - 21.

COMPOSITION OF THE LEAF PROTEIN SAMPLES.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Moisture %</th>
<th>Nitrogen % in dry matter</th>
<th>Protein % in dry matter, N x 6</th>
<th>LIPIDS % (in dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extraction with Chloroform:Methanol (2:1)</td>
</tr>
<tr>
<td>LPC</td>
<td>73.53</td>
<td>9.93</td>
<td>59.70</td>
<td>27.88</td>
</tr>
<tr>
<td>FD</td>
<td>6.51</td>
<td>10.00</td>
<td>60.00</td>
<td>27.19</td>
</tr>
<tr>
<td>OD₃</td>
<td>5.68</td>
<td>9.87</td>
<td>59.22</td>
<td>26.69</td>
</tr>
<tr>
<td>OD₂</td>
<td>5.85</td>
<td>9.84</td>
<td>59.04</td>
<td>26.50</td>
</tr>
<tr>
<td>OD₅</td>
<td>5.10</td>
<td>9.92</td>
<td>59.52</td>
<td>25.30</td>
</tr>
<tr>
<td>OD₄</td>
<td>5.09</td>
<td>9.91</td>
<td>59.45</td>
<td>24.20</td>
</tr>
<tr>
<td>RD</td>
<td>5.10</td>
<td>10.00</td>
<td>60.00</td>
<td>24.01</td>
</tr>
</tbody>
</table>
## TABLE - 22.

**Composition of the Solvent Extracted Leaf Protein Samples.**

<table>
<thead>
<tr>
<th>Samples extracted with Chloroform:Methanol (2:1) mixture</th>
<th>Samples extracted with Acetone solvent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Moisture %</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
</tr>
<tr>
<td>LPC&lt;sub&gt;a&lt;/sub&gt;</td>
<td>8.50</td>
</tr>
<tr>
<td>FD&lt;sub&gt;a&lt;/sub&gt;</td>
<td>8.12</td>
</tr>
<tr>
<td>OD&lt;sub&gt;1a&lt;/sub&gt;</td>
<td>7.87</td>
</tr>
<tr>
<td>OD&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>7.80</td>
</tr>
<tr>
<td>OD&lt;sub&gt;3a&lt;/sub&gt;</td>
<td>7.60</td>
</tr>
<tr>
<td>OD&lt;sub&gt;4a&lt;/sub&gt;</td>
<td>7.20</td>
</tr>
<tr>
<td>RD&lt;sub&gt;a&lt;/sub&gt;</td>
<td>7.20</td>
</tr>
</tbody>
</table>
Table 23, shows the extent of hydrolysis of several leaf protein samples by papain after 24 hours incubation. It also depicts the effects of heat treatment and solvent extraction on the hydrolysis of proteins by papain. The original material (LPC) and freeze dried sample (FD) were hydrolysed up to 68.4% and 67.90% respectively. The same samples when extracted with Chloroform:methanol (LPC\textsubscript{a} and FD\textsubscript{a}) or acetone solvent (LPC\textsubscript{b} and FD\textsubscript{b}) their papain digestibility was slightly increased. The dehydration of LPC by keeping it in oven at 70, 80, 90 and 100°C (OD\textsubscript{1}, OD\textsubscript{2}, OD\textsubscript{3}, and OD\textsubscript{4}) or by passing over steam heated rollers (RD) decreased the enzymic hydrolysis from 68.4 to 48.02%. The extraction of heated samples with Chloroform:Methanol (OD\textsubscript{1a}, to OD\textsubscript{4a} and RD) or Acetone solvent (OD\textsubscript{1b}, to OD\textsubscript{4b} and RD) almost restored the loss and the extent of hydrolysis with papain was measured from 65.1 to 66.98%.

Table 24 shows the net protein utilization (NPU) of the leaf protein diets fed to rats. The trends of NPU results were the same as those for papain in vitro digestibility. The NPU of rations containing LPC and FD samples was estimated to be 67.59% and 68.9% respectively. These samples when added into the diets after extraction with chloroform:methanol (LPC\textsubscript{a} and FD\textsubscript{a}) the NPU decreased to 64.95% and 64.51% respectively, however by extraction
TABLE 23.

PAPAIN IN VITRO DIGESTIBILITY OF THE LEAF PROTEIN SAMPLES AFTER 24 HOURS INCUBATION.

<table>
<thead>
<tr>
<th>Undefatted samples.</th>
<th>Samples extracted with Chloroform:Methanol (2:1) mixture.</th>
<th>Samples extracted with Acetone mixture.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Digestibility %</td>
<td>No.</td>
</tr>
<tr>
<td>LPC</td>
<td>68.40</td>
<td>LPC_a</td>
</tr>
<tr>
<td>FD</td>
<td>67.90</td>
<td>FD_a</td>
</tr>
<tr>
<td>OD_1</td>
<td>63.19</td>
<td>OD_1a</td>
</tr>
<tr>
<td>OD_2</td>
<td>55.02</td>
<td>OD_2a</td>
</tr>
<tr>
<td>OD_3</td>
<td>51.44</td>
<td>OD_3a</td>
</tr>
<tr>
<td>OD_4</td>
<td>48.02</td>
<td>OD_4a</td>
</tr>
<tr>
<td>RD</td>
<td>49.50</td>
<td>RD_a</td>
</tr>
<tr>
<td>No.</td>
<td>NPU %</td>
<td>No.</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>LPC</td>
<td>67.39</td>
<td>LPC_a</td>
</tr>
<tr>
<td>FD</td>
<td>68.00</td>
<td>FD_a</td>
</tr>
<tr>
<td>OD_1</td>
<td>63.32</td>
<td>OD_1a</td>
</tr>
<tr>
<td>OD_2</td>
<td>56.65</td>
<td>OD_2a</td>
</tr>
<tr>
<td>OD_3</td>
<td>50.35</td>
<td>OD_3a</td>
</tr>
<tr>
<td>OD_4</td>
<td>47.00</td>
<td>OD_4a</td>
</tr>
<tr>
<td>RD</td>
<td>50.30</td>
<td>RD_a</td>
</tr>
</tbody>
</table>
with acetone (LPC\textsubscript{1}, and FD\textsubscript{1}) the NPU was unaffected. The diets having heat-dried samples (OD\textsubscript{1} to OD\textsubscript{4} and RD) showed a progressive decrease in their NPU from 68.3 to 47.00\% and the decrease was proportional to the temperature and the time for which samples were heated. The loss in the nutritive value was largely reversed by the solvent extraction. The NPU of the heated samples after chloroform:methanol extraction (OD\textsubscript{1a} to OD\textsubscript{4a} and RD\textsubscript{a}) was noticed between 62.4 to 63.85\%. The values of the acetone treated leaf protein samples (OD\textsubscript{1b} to OD\textsubscript{4b} and RD\textsubscript{b}) were observed slightly higher and ranged from 65.72 to 68.91\%.

Table 25 shows the total amino acid content of the different leaf protein samples. The amounts of total leucine, isoleucine, valine, methionine, arginine, histidine, tryptophan, phenylalanine and threonine was observed almost the same in all preparations. The total lysine content however varied considerably. LPC and FD contained the maximum amount of lysine i.e. 7.65 and 7.55 g/16 gN, respectively. The samples OD\textsubscript{1} to OD\textsubscript{4} dried in oven at 70 - 100\(^o\)C and RD that dried, by passing over the steam heated rollers showed a decrease from 7.65 to 6.8 g/16 gN in the total lysine content. This loss in the total lysine from about 3 - 4\%, during the heating could neither be recovered by chloroform:methanol extraction nor
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Undesalted samples</th>
<th>Samples extracted with Chloroform-Methanol (2:1) mixture</th>
<th>Samples extracted with acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPC</td>
<td>FD</td>
<td>OD(_1)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.73</td>
<td>5.69</td>
<td>5.94</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.11</td>
<td>2.11</td>
<td>2.15</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.20</td>
<td>7.06</td>
<td>7.09</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.60</td>
<td>2.50</td>
<td>2.61</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>2.20</td>
<td>2.57</td>
<td>2.38</td>
</tr>
</tbody>
</table>

**Streptococcus zymogenes NCDO 522**

| Lysine           | 7.65   | 7.55  | 7.62     | 7.33     | 7.20     | 7.05     | 7.06     | 7.07     | 7.25     | 6.86     | 6.89     | 6.79     | 7.05     | 6.79     |

**Leuconostoc mesenteroides ATCC 8041**

| Threonine        | 5.28   | 5.30  | 5.29     | 5.21     | 5.23     | 5.29     | 5.26     | 5.24     | 5.30     | 5.20     | 5.30     | 5.27     | 5.29     | 5.27     |
by acetone solvent treatment.

Table 26 shows the amounts of available amino acid (g/16 gN) in several leaf protein samples. The quantities of available leucine, isoleucine, valine, methionine, arginine, histidine, tryptophane, lysine, phenylalanine and threonine estimated in LPC were 6.98, 4.0, 6.32, 1.95, 5.95, 2.19, 2.30, 5.50, 5.20 and 4.50 g/16 gN, respectively. The amount of available amino acids in freeze dried sample (FD) and solvent extracted samples (LPC_a, LPC_b, FD_a, and FD_b) were observed broadly similar to those found in LPC. The heat dried samples (OD_1 to OD_4 and RD) showed decrease in available leucine (7.6 to 24.1%), isoleucine (4.7 to 17.2%), valine (6.6 to 20.0%), methionine (7.5 to 25.0%), arginine (6.0 to 20.3%), histidine (6.4 to 22.4%), tryptophane (8.9 to 24.0%) lysine (10.0 to 28.7%), phenylalanine (7.6 to 25.0%) and threonine (4.4 to 27.0%), respectively.

Chloroform: methanol or acetone extraction of heated samples, resulted in a greater release of the bound amino acids with papain. The values of leucine, isoleucine, valine, methionine, arginine, histidine, tryptophane, phenylalanine and threonine measured after the solvent extraction ranged from 1.9 to 7.10 g/16 gN.

None of the combined lysine was released by the solvent treatment from the heated samples and the amount estimated was between 5.0 to 3.8 g/16 gN.
<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Undestroyed samples</th>
<th>Samples extracted with Chloroform-Methanol (2:1) mixture</th>
<th>Samples extracted with Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPC</td>
<td>PD</td>
<td>OD₁</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.50</td>
<td>5.40</td>
<td>5.00</td>
</tr>
<tr>
<td>Phosphatidyl</td>
<td>5.10</td>
<td>5.10</td>
<td>5.00</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.50</td>
<td>4.40</td>
<td>4.30</td>
</tr>
</tbody>
</table>
DISCUSSION.

The growth of plants is mainly dependent on the texture of soil and the amount of nutrients present in it, water supply, climatic conditions and the photosynthesis period (length of day). These growth factors have been considered in the present studies because in agriculture practice crop failure generally arise due to the lack of these requirements.

The profile discription (Table 1) and the mechanical cum chemical analysis (Table 2 & 3) of the soil showed that the tract selected for the experiments was adequately fertile. The profuse growth of Persian clover without the supply of any fertilizer also confirmed that the tract was naturally productive. The total yield of the foliage without the fertilizer treatment was estimated 86.59 Ton/hactare, where as the yield reported by Musahib-ud-Din (1966) was 84.65 Ton/hactare with ammonium phosphate fertilizer.

Effects of climatic variations and age on yield of the crop, its dry matter and nitrogen content, during the season.

The variations observed in the per hactare yield and nitrogen content of Persian clover in a season seemed to be due to the changes in the atmospheric temperature, relative humidity and the length of photosynthesis period.
The sudden decrease observed in the per hectare yield and nitrogen content of the 2nd and 3rd regrowths of 20 day and 1st regrowths of 30 and 40 day was due to the lowest atmospheric temperature (between 19.3 - 21.9°C max. and 3.1 - 5.7°C min.) and highest relative humidity (90% at 8.0 A.M. and 52-54% at 5.0 P.M.) recorded in the months of December and January (Table 4). In addition the shortest day (photosynthesis period), foggy weather and the frost which covered the plants were also responsible for the stunted growth of plants during these months. Although under these adverse conditions the yield and nitrogen content of the 80 and 100 day old 1st cuts were also affected, but since these cuts were not harvested during this period therefore the effect was not so evident. Musahib-ul-Din (1960) observed retarded growth in case of Persian and Egyptian clovers during the winter season i.e. in the months of December and January which supports our observation. The adverse effect of low temperature on the growth of plants was also noticed by Miller (1961) and it was attributed to decrease in the absorption of nutrients and water from the soil by the plants. Batham and Nigham (1930) reported that nitrogen content of the soil was lower in winter than in summer because of low temperature the biological activities slowed down. Moreover the greater leaching of nitrates occurred in winter which kept the nitrogen supply
low. Miller (1938) observed that in most of the plants the activity of the enzymes decreased when the temperature fell below 37°C. A proportionate decrease in the rate of transpiration of plants with an increase in the relative humidity was reported by Darwin (1914). Garner and Allard (1920) observed that some plants become weakly vegetative when the length of days was short because the quantity of carbohydrates manufactured decreased which in turn affected the nitrogen supply. The unfavourable effects of low temperature, short days and deficiency of nitrogen on the vegetative growth of potato plants were also studied by Warner (1934). Gibson (1966) noticed that at 5-10°C Trifolium subterraneum retained most of the nitrogen in roots and its translocation to shoots was retarded.

The above findings indicated that low atmospheric temperature, high relative humidity and the short length of day (photosynthesis period) were the factors mainly responsible for the stunt growth of Persian clover in the winter season.

A rise in an atmospheric temperature from 19.3 to 27.8°C max. 5.1 to 13.1°C min., fall in the relative humidity from 90 to 66% (at 8.0 A.M.) and 56 to 33% (at 5.0 p.m.) and a moderate increase in the length of the day in the months of February and March enhanced the yield/haectar of the crop and its nitrogen content. It is
evident, that in the 4th & 5th regrowths of 20 day, 3rd & 4th regrowths of 30 day and 2nd regrowth of 40 day the yield/hectare of fresh and dry weights and nitrogen content increased progressively (Fig. 2-1). More over the 60 and 80 day old 1st regrowths and the 100 day old 1st cut also gave the better yields (fresh and dry weights) and nitrogen content than their respective cuts and regrowths (Fig. 5-7). These changes were due to the favourable climatic conditions and photosynthesis periods.

Further increase in atmospheric temperature (from 27.8 to 40.2°C max. 13.1 to 24.0°C min.,) which was associated with a proportionate decrease in the relative humidity (from 66 to 41% at 8.0 A.M. and 35 to 22% at 5.0 P.M.) in the months of April and May (Table 1) proved determinental to the growth of the crop. This finding was based on the fact that the per hectare yield (fresh and dry weight basis) and the nitrogen content remarkably decreased in 20 day old 6th - 8th regrowths, 30 day old 5th & 6th regrowths, 40 day old 2nd & 3rd regrowths, 50 day old 2nd regrowths and 100 day old 1st regrowth (Fig. 2-7) that were harvested in these two months. The effects of climatic conditions on the crop was further supported by the fact that growth of plants completely ceased in the month of June when atmospheric temperature was the highest (41°C max. and 26.8°C min.), relative humidity was the lowest (40% at 8.0 A.M. and 22% at 5.0 P.M.) and the days were longest (Fig. 5).
This continuous decrease in the yield and nitrogen content of regrowths in the last two months of the season could be attributed to the loss of excess of water from the stems and leaves over the amount absorbed by the roots. The wilting and high dry matter content of plants supported this assumption. The death of Persian clover after May was basically due to the genetic constitution apart from these external factors cited above. Persian clover is a seasonal crop and the plants die after producing seeds. The experimental evidence given by Miller the (1938) agreed with these views. Leitch (1916) reported that the fall in the growth of plants under the influence of higher temperature for long periods could be due to the decrease in rate of enzymes action and photosynthesis. Reed (1919) reported that the growth of plants was dependent not only on the external factors but also on its genetic constitution.

The above findings confirmed that the wide variations observed in the per bactare yield (fresh and dry weight basis) and nitrogen content of the different regrowths of Persian clover were due to changes in atmospheric temperature, relative humidity and the length of days during the season.

The harvesting interval between the successive regrowths or the age of crop was another most important factor which directly affected the total yield of the crop.
It is evident from the results that harvesting of the crop after an interval of 20, 30, 40, 60, 80 and 100 days gave different yield/ha/season of fresh and dry weights and nitrogen content (Fig. 8). The statistical analysis of the data, in the table 6 also showed highly significant variations among the total yields of the crop, when harvested after 20 to 100 days intervals (Table 8). Definitely the major factor inducing these differences in the yield was the age factor.

Comparatively less yield/ha/season obtained from the 20 day old 1st cut and regrowths, seemed to be due to harvesting of the crop too frequently. Masahib-ud-Din (1960) noticed in case of Egyptian clover (Barseem) that too frequent cuttings devitalised the plants which adversely affected the total yield. The highest total yield obtained from 30 day old cut and regrowths showed that an optimum interval between the successive cuttings was 30 days, in addition, it supported that the factor which entailed some loss in the total yield of the 20 day old cut and regrowth was the harvesting of crop at the immature stage. The least significance difference test applied to mean total yield; (Table 9) showed an insignificant difference among the mean total yields of 20, 30 and 40 day old 1st cuts and regrowths. But there were significant variations among the mean total yields of 60 - 100 days old cuts and regrowths (Fig. 10).
The conclusion of this statistical analysis therefore was, that yield/hectare/season (total yield) of Persian clover was almost the same when the interval between the successive cuttings was 20, 30 and 40 days. The slight variations noticed in the total yield of 20-40 day old cuts and regrowths were just due to a chance. But the continuous decrease observed in the total yields of 60, 80 and 100 day old cuts and regrowths was real and it was definitely due to increase in the age of the crop.

Musahib-ad-Din (1966) suggested five cuts of Persian clover from 10th November to 15th May, for having the maximum yield of green fodder i.e. 87.65 Ton/hactare. However in the present investigations the maximum total yield of the clover, obtained from Persian clover was 86.59 Ton/hactare by harvesting it six times over the same period.

The presence of maximum amount of total nitrogen content (0.438 Ton/hactare/season) in the 20 day old 1st cut and regrowths (Fig. 8) was based on the fact, that the plants were young, and contained higher percentage of nitrogen on the dry matter basis than all the other cuts and regrowths. (Fig. 9). But in the 30, 40, 60, 80 and 100 day old 1st cuts and regrowths the percentage of nitrogen decreased with the age (Fig. 9) therefore the yield/hectare/season of nitrogen content also decreased in the same proportion. This decrease in nitrogen content of
the different cuts and regrowths at various stages of maturity also seems to be due to an increase in fibrous tissue. The investigation of Miller (1965) that a decrease in the nitrogen content in Rye plants at maturity was due to increase in the percentage of lignin pantosans and cellulose supported the above views.

Effects of climatic variations and age on the yield of extractable nitrogen, extractable protein nitrogen and some other fractions of the crop during the season.

The total nitrogen (TN) in the pulp, extractable nitrogen (EN) and extractable protein nitrogen (PN) in the juice of the crop was also affected considerably by the changes in climatic conditions and age like the yield of crop.

The yield of TN, EN and PN seemed to be adversely affected by the atmospheric temperature between 19.3 - 21.9°C max. and 3.1 - 5.7°C min. when the relative humidity was 90% at 8.0 A.M. and 54% at 5.0 P.M. in the month of December and January. (Table 4). Those observations were based on the results that yield/hectare of these constituents decreased in the 20 day old 2nd & 3rd regrowths, 30 day old 2nd regrowth and 40 day old 1st regrowth harvested during this period (Fig. 11-13). The percent yield of EN and PN also showed the same trend although TN(%) increased slightly. The decrease noticed in EN was from 52.8 to 56.02% whereas in PN was from 45.14 to 38.9% respectively in these regrowths (Table 11).

An increase in atmospheric temperature from 19.3 - 27.8°C max. and 3.1 - 13.1°C min. was accompanied by a
decrease in relative humidity from 90 to 66% at 8.0 A.M. and 54 to 35% at 5.0 P.M. (February to March) favourably affected the yield of TN, EN and PN of the crop. The continuous increase observed in kg/hectare yield of these constituents in the 20 to 60 day old regrowths seems to be due to these changes in temperature and relative humidity. The 20 day old 5th regrowth, 30 day old 3rd regrowth, 40 day old 2nd regrowth and 60 day old 1st regrowth, harvested in the month of March (just before the flower initiation) gave comparatively more yield of TN, EN, and PN than their respective cuts and regrowths, simply because these regrowths were harvested at the time when the climatic conditions were most favourable. (Fig. 10-11). During this period the per hectare yield of these constituents increased progressively with an increase in the age of the crop. It was due to the same reason that yield of TN, EN and PN increased from 28.1 to 91.1, 17.8 to 39.0 and 11.7 to 23.0 kg/hectare respectively in the 20 to 160 days old 1st cuts (Fig. 11-16). The percent yield of EN, and PN, varied negligibly upto the age of 60 days, but percentage of TN showed a slight decrease. These observations could be supported by the results (Table 11 & 12) that the amount of EN and PN remained between 50-54% and 10-15% in the 20-60 day old cuts and regrowths harvested in the months of February and March. The percentage of EN and PN however decreased after the age of 60 days in this period. It is
evident that amount of EN and PN first decreased to 49.1% and 32.3% respectively in the 80 day old 1st cut and then reduced to 12.67% and 25.67% respectively in 100 day old 1st cut. This decrease in the percentage of EN and PN at the age of 80 and 100 days seemed to be due to the age factor. Chibnall (1922) observed decrease in extractability of nitrogen in runner bean leaves with an increase in the age. Such decrease in the extractable total nitrogen and protein nitrogen content of the plants with an increase in the age was also reported by Pleshkov and Fowden (1959). The findings of Byers and Sturrock (1965) were also the same.

An increase in an atmospheric temperature from 27.8 - 40.2°C max. and 13.1 to 23.6°C min. during the months of April and May, was accompanied by a decrease in relative humidity from 66 to 41% at 8.0 A.M., and 35 to 22% at 5.0 P.M. This resulted in a rapid decrease in the yield of TN, EN and PN of the crop. The decrease noticed in Kg/haectare yield of these constituents in the 6th - 8th regrowths of 20 day, 4th & 5th regrowths of 30 day, 3rd & 4th regrowths of 40 day, 2nd regrowth of 60 day, 2nd regrowth of 80 day and 1st regrowth of 100 day was due to these climatic changes. (Fig. 11-16). This decrease in the yield of TN, EN and PN in the months of April and May was not only due to the adverse climatic conditions but it also as a result of an increase in the age of crop. This finding is supported
by the results that amount of these fractions in the 20
day old last regrowth was 3.10, 38.0% and 23.41% which
decreased to 2.0, 25.0 and 1.5% respectively in the
100 day old last regrowth (Table 11).

This continuous increase in atmospheric tempera-
ture and decrease in relative humidity during the last
two months of the season i.e., April and May, resulted in
a progressive increase in dry matter content of the plants
because of the water evaporation from the stems and
leaves. These changes were also associated with the
decrease in nitrogen content and an increase in the fibrous
tissue of the plants. Most of the regrowths were mature and the
phenomena of flower and seed development were in progress.
It was probably due to these metamorphosis in plants the
yield of TN, EN and PN was adversely affected in these
last two months of the season. Some of the regrowths such
as 60, 80 and 100 day old last regrowths harvested in the
month of May were extremely fibrous, almost dry, turning
to yellow and due to that the proteins were poorly extrac-
ted from these regrowths. Crook and Holden (1948)
reported that extraction of proteins from leaves was
mainly influenced by their nitrogen content and dry matter.
Morrison and Pirie (1961) observed a decrease in percent
extraction of proteins at the maturity of the plants and
extracted only 10 to 25% of the nitrogen content with
water or alkali from the leaves turning to yellow. Sing
(1961) suggested that poor extraction rate of proteins from matured leaves with high dry matter content was probably due to an increase in fibrous tissue and decrease in the fragility of ageing chloroplasts which caused greater retention of matured chloroplasts in an increased amount of fibrous tissue.

The above findings clearly indicated that changes in the yield of TN, EN and PN were mainly due to the variations in climatic conditions and age of the crop. Apart from that the incomplete disintegration of fibrous tissue at maturity of the crop was also responsible for the decrease in extractable nitrogen. The maximum yield of these fractions in Persian clover was noticed in the month of March i.e. before the flower initiation. Birtie (1971) reported that the climatic conditions and the age of the crop had larger effect on the yield of extractable proteins. The maximum proteins could be obtained from the crop by harvesting it just before the end of vegetative growth and at the start of flower growth. Byers and Sturrock (1965) observed similar changes in the extractability of protein from wheat and rye plants. Extraction of proteins from these plants increased with an increase in the age but decreased at the start of flower initiation. These findings fully support our observations.

The protein nitrogen (PN) precipitated by heating the juice at 80°C was observed comparatively less than
the TCA precipitable nitrogen in almost all the cuts and regrowths. (Table 11 & 12). The same were the findings of Slade et al. (1979) and was reported that TCA precipitates contained about 10 to 25% more nitrogen than heat coagulable of the similar extract. Singh (1968) supported these views and gave the reason that TCA solution precipitated more nucleic acid from the extract than that precipitated by heating at 80°C.

The changes noticed in the yield/hectare/season of the TN, EN and PN of the crop on harvesting it often an intervals of 20-100 days seemed to be due to the age factor. An increase in the age of crop resulted in a decrease in the yield/hectare/season of these fractions. It is evident from fig. 16 that decrease in TN, EN and PN was from 528.1 to 392.5, 247 to 31.3 and 130.2 to 22.5 kg/hectare/season respectively when an increase in the intervals between the harvests (cuts and regrowths) was from 20-100 days. The comparatively more proteins (900 kg/hectare/season) obtained from the 30 day old 1st cut and regrowths was due to the fact that the crop was lush, contained more nitrogen and lesser fibrous tissue than 30-100 day old cuts and regrowths. The previous work done at Rothamssted Experimental station (Bvers 1961) supports these findings that more proteins could be extracted from the young foliage.

The non-proteinous nitrogen (NPN) in the
Juice increased with an increase in the age of crop and atmospheric temperature. Such changes in NPN were more rapid during reproduction. This increase in the NPN in the juice (Table 1) seemed to be due to the presence of proteolytic enzymes in plants, which hydrolysed the proteins. Previous work done by Nazir and Shah (1968) showed that juice extracted from Persian clover definitely contained proteolytic enzymes. Singh (1962) observed an increase in the activity of proteolytic enzymes during the reproductive stage in wheat plants. Probably due to the same reason the NPN in Persian clover increased more rapidly in the juice of the regrowths, taken in a period when flower and seed development was in progress. Kolonsek and Coulson (1935) reported that the increase in NPN fraction after budding was due to the protein degradation, because for the process of flowering, simple nitrogenous materials were required. The other reason for the rapid increase in NPN during the last two months of the season could be the increase in atmospheric temperature which increased the rate of protein autolysis in the juice during extraction.

The yield of fibrous residue (FR) was also affected by the climatic variation and age of the crop. The increase observed in the FR from 33.0% to 87.84% (Table 15 & 16) in the 20-100 day old crop and regrowths was due to the changes in the same factor. The nitrogen
content of FR also showed considerable variations and the increase or decrease observed in it was proportional to nitrogen content of the crop. The FR obtained from the young foliage contained more nitrogen than the FR from the older and matured crops. It was reported by Byers and Sturrock (1965) that nitrogen content of FR tends to increase in proportion to the nitrogen content of leaves. The nitrogen content of fibre in the young plants of cereals was reported 3% which decreased to less than 1% in some late cuts of the same species. The nitrogen content of FR in different cuts and regrowths of Persian clover also showed similar changes. The FR obtained from 20 day old first, 30 day old first five, 40 day old first four and 60 day old first two harvests contained from 3.0 to 4.28% nitrogen. The amount of nitrogen decreased in the FR of some late harvests (20, 30, 40 and 60 day old last regrowths, 80 and 100 day old first cuts & regrowths) and was observed between 1.6 to 2.83% (Table 15 & 16). The total yield of FR and its nitrogen content seemed to be dependent on the yield of crop. It is evident from Fig. 17 that yield of FR in the 20, 30 & 40 day old harvests (cuts & regrowths) was comparatively more (5730.7 to 6412.3 Kg/hectare/season) than 60-100 day old cuts & regrowths because in the former harvests the yield of crop
was also more. Similarly the un-extractable nitrogen in the FR of these harvests was also more, i.e. between 179.9 to 200.7 Kg/ha/acre/season. These results indicated that FR obtained from 20 to 40 day old cuts and regrowths was not only abundant in quantity but also contained 15.84 to 21.01% proteins. This by-product could be recommended for animal feeding. Chipmull (1922) reported that unextracted proteins in the FR were of the same quality as those of extracted proteins. The digestibility trials with sheep carried out in New Zealand and some other places showed that fibrous residue, either vacuum packed, silaged, dried or fresh had satisfactory digestibility.

The decrease noticed in the nitrogen content of LPC that prepared at the end of season from last regrowths (20 day old 8th regrowth, 30 day old 5th regrowth, 40 day old 4th regrowth, 60 and 80 day old 2nd regrowth, and 100 day old 1st regrowth) could be due to the maturity of the crop. Such decrease in the nitrogen content of leaf protein preparations from the matured crop was also observed by Morrison and Pirie (1961). A decrease in water soluble substances, and nitrogen content and an increase in pantothen, cellulose and lignin in the matured Rye plants was reported by Miller (1965). So the decrease noticed in the nitrogen content of LPC at the end of season could be attributed to the maturity of plants employed.
The maximum yield of LPC was 1500 Kg/hactare/season from Persian clover and the protein content of the product was 900 Kg (60%) (Table 14). Pirie (1971) reported that leguminous crops such as lucerne and Red clover usually yield about 1000 Kg protein/hactare/year. The reason for the comparatively less protein yield in case of Persian clover, could be the differences in the species, climatic conditions and employing a different apparatus for the protein extraction. More over the period of growth was also lesser in the case of Persian clover. The yield/season of proteins from the crop could be enhanced by the second extraction after addition of water but it was not carried out. Because the main object was to extract only the easily extractable portion of proteins and to leave the rest in the FR for the animal feeding.

The volume of waste liquor obtained from 20 to 100 day old cuts and regrowth in a season also varied considerably and same was the case with its nitrogen content. The total yield of the waste liquor and the amount of uncoagulable nitrogen present in it seemed to decrease with an increase in the age of crop. It is evident from table 17 that total yield of waste liquor (litre/hactare/season) in 20 to 40 day old harvests (cuts & regrowths) was more than that from 60 to 100 day old harvests processed in a season, and same was the case with un-
coagulable nitrogen present in the waste liquor. These results also indicated that variations in the total yield of waste liquor and the amount of uncoagulable nitrogen present in it was dependent on the volume of juice extracted from the crop and its XPN content.

The chemical analysis of the waste liquor (Table 18 & 19) indicated that it contained sufficient nutrients and could be used as a substrate for the growth of micro-organisms. It was reported by Pirie (1971) that uncoagulated material in the juice of plants was a good culture medium for the micro-organisms. Byers and Sturrock (1965) observed that solubile fraction (XPN) of the steamed extract contained amino acids, nucleotides, inorganic nitrogen, large amount of carbohydrates and it could be used as substrate for growing micro-organisms. A satisfactory propagation of Candida utilis (Brewer's yeast) in the waste liquor of Persian clover supported the above views (Table 20). Johnson (1962) observed a satisfactory growth of Rhizobium meliloti, Penicillium chrysogenum, and Aspergillus niger, in "khey" (waste liquor) from pea vines and other leafy materials. More recently but et al. (1972) also observed a successful growth of nine yeast strains in the supernatant obtained after the heat precipitation of protein from the juice of three different species.
It can be concluded from the foregoing findings that yield of extractable proteins and other fractions varied considerably in a season due to changes in climatic conditions, age and the maturity of the crop. The regrowths taken in the month of March, just before the initiation of floral growth contained more extractable proteins than their 1st cut and respective regrowths. Harvesting of the crop regularly after an interval of 20 days gave maximum yield of extractable proteins i.e. 900 Kg/hactare/season. An increase in the age of the crop from 20-40 days although increased the per hectare yield of FR and waste liquor but it decreased the yield of extractable proteins. Further increase in the age of crop resulted in a decrease in yield/hactare/season of FR and waste liquor. The FR obtained from 20-40 day old harvests (cuts & regrowths) was in abundant quantity, contained sufficient proteins and could be utilised as a feed for ruminents. The waste liquor was a good culture medium for the propagation of Candida utilis (food yeast). The maximum out put of proteins from a unit area of land by growing Persian clover and utilization of waste liquor for protein synthesis was about 1078 Kg/hactare/season.

Effects of different methods of drying and solvents extraction on the nutritive value of leaf protein concentrate.

The protein co-agulum (LPC) was stored in defreeze at -20°C to protect it from staling and various attempts were made to prepare a fairly stable, convenient in handling
and a highly nutritive product from it. The treatments like dehydration and solvent extraction of LPC were carried out for the same purpose.

The dehydration of leaf protein by heat treatment resulted in a decrease in the extractability of lipids. The decrease noticed in this case was according to an increase in temperature and the time allocated for heating. It is evident from table 21 that lipids extracted from heated samples (OD₁ to OD₄ and RB) were lesser than those of unheated products. More over a progressive decrease was noticed in the percentage of lipids extracted with chloroform:methanol mixture or acetone from the samples dried in an oven at 70 - 100°C or by passing over steam heated rollers. The decrease in the extractability of lipids could be due to oxidation of free lipids and formation of insoluble complexes by phospholipids and oxidation products of the lipids with proteins as reported by Shah (1971). The oxidation of lipids present in wheat leaf proteins on storing at various temperatures and the resultant decrease in the extractability of lipids was also discussed by Buchanan (1969,a).

Chloroform:methanol (7:1) mixture was a better solvent for the extraction of lipids from leaf proteins than acetone. The results in table 21 showed that lipids extracted with chloroform:methanol mixture from different preparations were from about 3 to 1½ more than that of
acetone. The reason for it could be that chloroform:
methanol extracted phospholipids and some non-lipid
fractions that were insoluble in acetone solvent. It
was reported by Lester and Fleischer (1961) and Weenink
(1961) that acetone was a poor solvent for phospholipids
and the lipids extracted by it from leafy materials
consisted of largely galactoolipids. Buchanan (1969,b)
observed that chloroform removed the sterol esters, tri-
glycerides, free fatty acids, 1:3 and 1:2 diglycerides, free
sterols and monogalactosyl diglycerides, steroles glycosides
phosphatidyl ethanolamine, digalactosyl diglyceride and
sulpholipids from heated proteins. Naturally chloroform:
methanol could have extracted all such fractions and also
some non-lipid material released by methanol.

The nutritive value of the LPC was affected by
heat treatment and the damage caused to proteins was direc-
tly proportional to the temperature and the length of
heating. The losses in the nutritive value of proteins
were detected by both the in vitro and in vivo tests. The
results set out in Table 23 showed that papain in vitro
digestibility of the samples dried in oven at 70 - 100°C
(OD₁ to OD₂) or by passing over heated rollers (RD)
decreased from 68.0 to 48.00%. Similarly the NPU of these
samples fell from 68.0 to 47.00%. The reason for it could
be the formation of protein complexes with lipids. Buchanan
(1969,b) reported that losses in TD and enzymic solubility
of protein on heating were due to the formation of protein complexes with unsaturated fatty acids like linolenic and linoleic acids. But according to Lea et al. (1960) the deterioration in nutritive value of proteins on storing at room temperature or mild heating was predominantly oxidative and on severe heating was non-oxidative type. Bieły et al. (1951) also reported losses in the nutritive value of whole herring meal on heating at 149°C due to oxidation of lipids. Miller (1956) attributed the loss in nutritive value of strongly heated fish meals to carbonyl-amine (Maillard) reaction.

The greater differences in the amounts of available amino acids in the heated and unheated products also reflected the losses in the nutritive value of proteins. It is evident from the results of microbiological tests (Table 26) that availability of leucine, isoleucine, valine, methionine, arginine, histidine, tryptophane, lysine, phenylalanine and threonine decreased by 17.2 to 28.7% during drying. The reason for this decrease in availability of amino acids in heated samples (OH, to OD, and RD) could be formation of such protein complexes which were not susceptible to the action of proteolytic enzymes. Ford (1962) reported the formation of a tangle of intramolecular linkages, not amenable to enzymic hydrolysies, involving ε-amino groups of lysine moieties distributed throughout the peptide chain, Harris and
Mettill (1940) observed the formation of indigestible compounds like diketopiprazine due to complexing of free amino groups with carboxylic group. Mecham and Olectic (1947) reported that decrease in amino nitrogen and rate of digestion by enzymes on dehydration of proteins could be due to the loss of polar group by internal formation of amides or esters. Pierpoint (1960 a,b) reported that o-quinones and polyphenols also react with ε-amino group of lysine and it could be responsible for loss in availability of amino acids. Yanez et al (1970) reported formation of enzyme resistant bonds between ε-amino group and carboxylic group from monosaccharides while studying the effects of drying temperatures on the availability of amino acids from fish proteins.

Chemical hydrolysis broken down the complexes formed during heating of proteins and released all bound amino acids that could not be liberated by enzymatic hydrolysis (in vitro) or by digestion in vivo. A small portion of lysine seemed to be destroyed during heating. Probably due to the same reason the unheated and heated proteins when hydrolysed chemically the resulting amino acid mixture differed in lysine content but were alike in composition with respect to other amino acids. The decrease observed in the lysine content of the samples dried in oven at 70 - 100ºC (O1 to O4) and roller dried sample (RD) was from about 5.6 to 11.1% (Table 25). Lea et al (1960) while
studying the chemical and nutritional changes in herring meal also observed destruction of lysine during the reaction between fat, oxidation products and proteins. Chemical hydrolysis was observed unsatisfactory because it failed to take into account the digestibility and biological availability that were of paramount importance to evaluate the heated food stuffs.

The solvent extraction of the heated leaf proteins strikingly increased their nutritive value. It is evident from the results in table 23 that papain digestibility of the heated samples after extraction with chloroform, methanol (OD$_{1a}$ to OD$_{4a}$ and RD$_{a}$) or acetone (OD$_{1b}$ to OD$_{4b}$ and RD$_{b}$) increased as much as the decrease occurred during heat processing. The in vivo criteria i.e., XPU and available amino acid estimations used for protein evaluation reflected the same trend as the papain digestion. The results indicated that after solvent extraction papain could eventually break down the lipid protein complexes. It was reported by Buchanan (1969) that losses in enzymic solubility, BV, DP, NPU and PER on heating of LPC could be largely reversed by mild solvent extraction. The papain solubility of Naiz leaf protein as measured by Byers (1967) also increased by extraction of lipids with solvents.

A close similarity was noticed in the extent of hydrolysis with papain and availability of amino acids in
the protein sample extracted with chloroform:methanol mixture or acetone (Table 23). It indicated that lipids left after extraction with acetone did not affect the enzymic hydrolysis and the physical barrier between the enzymes and protein was removed effectively by acetone.

The NPU of all the leaf protein samples defatted with chloroform: methanol mixture was comparatively lesser than those extracted with acetone (Table 24), inspite of the fact that the former had extracted more lipids. The reason for it could be that chloroform: methanol also removed some proteolipids proteins or non-lipids soluble fraction like simple peptides from the samples as reported by Siah et al (1967).

It was concluded from above findings that dehydration of IPC by freeze drying did not affect the nutritive quality of the proteins. Drying of IPC over hot air at 70 - 100°C in oven or by passing over the steam heated rollers considerably decreased the extractability of lipids, papain in vitro digestibility, NPU and amino acids availability. These losses in the nutritive value of leaf protein caused by heating could be recovered by extraction of the samples with chloroform: methanol, or acetone solvent. The leaf protein cake, products dried over hot air at 80 ± 2°C and freeze dried sample after extraction with acetone (IPE, FIV, DP, and DO2) showed
better digestibility with papain, net protein utilization and amino acid availability than the other preparations. These products can be stored at room temperature and are recommended for supplementing the protein deficient diets of human beings.
SUMMARY

1. Yield and composition of Persian clover varied during the season.

2. Growth of the crop and its protein content was noticed maximum when the atmospheric temperature was between 24.4 to 27.9°C Max. and 6.0 to 15.1°C Minimum.

3. Harvesting intervals also affected the yield and composition of the crop. The yield of the crop was maximum (96.59 ton/hactare/season) when it was harvested after an interval of 30 days.

4. Maximum yield of the extractable proteins, 900 kg/hactare/season was obtained from the 20 day old 1st cut and regrowths.

5. Fibre content of the crop increased while its nitrogen content decreased with an increase in the age.

6. The filtrate (waste liquor) left after heat co-agulation of proteins from the juice of the crop was successfully employed for the propagation of Candida utilis.

7. Maximum yield of proteins in a season from a unit area of land including the yeast proteins obtained from the waste liquor was estimated to be 1078 kg/hactare.

8. Nutritive value of leaf protein concentrate (LPC) was least affected when it was freeze dried or extracted with solvents.
9. Dehydration of LPC by heat treatment reduced the in vitro and in vivo digestibility and amino acid availability.

10. Removal of lipids and oxidation products from the leaf proteins improved their nutritive value.
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APPENDIX

[Nature of Image]

1. Photo 1
2. Photo 2
3. Photo 3

[Further Text]

1. Additional information
2. Relevant details
3. Conclusion