Protein Juice From Three Forage Legumes

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ABSTRACT

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The production, processing and storage of protein-rich juice extracted from Ladino clover (<u>Trifolium repens</u> L.) was evaluated for possible use as a protein supplement in swine rations.

Dry matter yield of fresh forage material, juice yield from this forage material, as well as yield of protein concentrate from the juice through a fermentation process were recorded from plots with six successive first cut dates and recut at twenty-eight day intervals thereafter. Processing of pre-bloom forage material proved to yield highest amounts of protein concentrate.

Yields of all fractions involved in processing forage leaf material, from red clover (<u>Trifolium pratense</u> cv. Ottawa), alfalfa (<u>Medicago sativa</u> cv. Iroquois) and Ladino clover cut at 1/10 bloom were compared. No significant differences in seasonal yield of protein/concentrate per unit area among these species was observed.

Additives, including acetic acid and molasses, were useful in prolonging storage life of leaf protein material. Both methods were equally effective for storage purposes but immediate coagulation of protein on the addition of acetic acid resulted in the greatest yield of protein concentrate.

Many problems associated with the production, processing and storage of protein-rich juice extracted from Ladino clover are evident from this project. Protein concentrate from Ladino clover cannot be recommended for use as a protein supplement in swine rations at this time.

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La production, la transformation et l'entreposage de jus riche en protéines extrait du trèfle Ladino (<u>Trifolium repens</u> L.) a été évalué pour une utilisation possible comme un supplément de protéine dans des rations pour porcs.

La production de matière sèche de ce matériel de fourrage frais, la production de jus de ce matériel de fourrage, ainsi que la production de concentré de protéine du jus par un procédé de fermentation furent enregistrés à partir de lots avec six dates successives de première coupe et de recoupes à des intervalles de 28 jours par la suite. La transformation de matériel de fourrage avant sa floraison s'est avérée produire une plus grande quantité de concentré de protéine.

On a comparé la production de toutes fractions impliquées dans la transformation des feuilles de fourrage, à partir de trèfle rouge (<u>Trifolium pratense</u>, cv. Ottawa), de luzerne (<u>Medicago sativa</u>, cv. Iroquois) et de trèfle Ladino coupe à 1/10 de sa floraison. On a remarqué aucune différencé significative dans la production saisonnière de concentré de protéine par unité d'aire parmi ces espèces.

Les additifs, y compris l'acide acétique et la mélasse, étaient utiles pour prolonger la vie d'entreposage des matières de protéines de feuilles. Les deux méthodes étaient aussi efficaces pour fin d'entreposage mais une coagulation immédiate de protéines avec l'addition de l'acide acétique a résulté à la plus grande production de concentré de protéines.

Plusieurs problèmes associés a la production, la transformation et l'entreposage de jus riche en protéines extrait du trèfle Ladino sont évidents dans ce projet. On ne peut recommander le concentré de protéines du trèfle Ladino pour utilisation comme un supplément de protéine dans les rations pour porcs à ce moment.

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INTRODUCTION

The production of high quality home-grown protein for supplementation of swine diets is one goal of farmers in the Atlantic Provinces of Canada. Because of the climate, soybeans, which are commonly used as a protein supplement, are not a reliable crop. Forages, on the other hand, grow and produce well under the cool, moist conditions of the region.

The development of a system whereby forage material is fractionated into a protein-rich juice, for use as a supplement in swine rations, and pulp, for ruminant feed, is therefore of interest.

Trends indicate that the yield of protein concentrate from forage decreases with increasing maturity (Lee and Smith 1972). An experiment was therefore designed in an attempt to establish the growth stage at which forage material should be cut to yield the greatest amount of protein through the fractionation process. Ladino clover was the major species used in this experiment although some work was carried out with red clover and alfalfa.

Once the fractionation process is complete, juice must be used quickly, or further processed, as chemical reactions occurring soon after expression of the juice cause rapid deterioration evidenced by a change in color and odor as well as a thickening of the juice. The need for protein for diet supplementation during the winter months when fresh material is not available necessitates the long-term storage of leaf protein. Both fermentation (Anonymous 1977) and addition of organic acids (Arkcoll 1973) have been suggested as treatments to prevent spoilage.

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A third experiment was then devised to test and compare fermentation, with and without the addition of molasses, and the addition of organic acids as methods of storing the protein-rich forage juice. Such processes concentrate the leaf protein through coagulation therefore yields of protein concentrate attained by various treatments were compared.

In summary, the goals of this project were: (1) to determine the growth stage at which Ladino clover (<u>Trifolium repens</u> L.) yields the greatest amount of leaf protein through fractionation, (2) comparison of yield of protein concentrate using alfalfa, red clover and Ladino clover, (3) to evaluate several methods of long-term storage of the leaf juice protein.

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LITERATURE REVIEW

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Green Crop Fractionation

Green crop fractionation is a process in which green leaf material is separated into a protein-rich juice and a fibrous pulp material. The protein juice may be fed directly to non-ruminant animals or further processed for use as food or feed. The pulp residue can be fed to ruminants either directly or after drying or ensiling.



and fermentation process

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Species For Fractionation

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Most crop plants used in agriculture have been selected according to criteria such as the ability to yield abundant seed or the rapid production of dry matter but these characteristics may well be adverse to production of leaf protein. Pirie (1978) suggests that species most useful for leaf protein production will probably have flowering delayed or prevented: by sowing at unusual times or in unusual latitudes; by genetic manipulation; or through the use of growth regulators so that senescence is delayed and there is a prolonged period of vegetative growth.

Crops for use in a fractionation process should produce abundant, lush, protein-rich leaves. Leaves should not be carried on a very fibrous stalk as the energy needed for pulping will be excessive (Pirie 1978). The leaf should be neutral or slightly alkaline although acidity can be partly counteracted by pulping with alkali. The presence of tannins and phenolic substances diminishes protein extractability.

Possible sources of leaf material are grouped into the following categories by Pirie (1978):

- Conventional species such as: wheat, ryegrass, alfalfa and clovers;
- (2) Leaves; available as the by-product of a conventional crop,such as: sugar beet tops, potato tops and pea vines;

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(3) Tree leaves: little work has been done on this aspect although a food-producing tree crop would be ideal in tropical rain , forests;

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(4) Water weeds such as: water hyacinth;

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(5) Miscellaneous: unconventional species such as mustard.

Ladino clover (<u>Trifolium repens</u> L.) is a large form of common white clover which originated in Italy and is now widely distributed. (Ahlgren, G. H. <u>et al.</u> 1950). The most favourable habitat is a moist, cool region in which growth is continuous. It will withstand greater temperature extremes than either red or alsike clovers. It is adapted to moist soils, especially clays and loams, which are abundantly supplied with phosphorous and potash. Growth is not good on strongly acid or alkaline soils. (Martin et al. 1975).

Botanically, white clover is a perennial of widely different forms with prostrate growth habit. Seedlings have a rosette type of leaf growth and a small crown from which stolons develop. Stems root at the nodes. Roots are generally shallow. Ladino type, except for seed, is two to four times as large as common white.

White clover is one of the most nutritious and palatable of all legumes as only leaves and flowers are harvested.

Nutritive values differ widely at different stages of maturity and are influenced by cultural practices and location. It varies less in nutritive value than other legumes where stems are harvested as part of the forage? (Heath <u>et al.</u> 1973).

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Alfalfa (<u>Medicago sativa</u>) which is referred to as lucerne in Europe is believed to have originated in southwestern Asia. As shown by its wide distribution, alfalfa has a remarkable adaptability to various climatic and soil conditions.(Martin <u>et al.</u> 1975).

The alfalfa plant makes its best growth in relatively dry climates where water is available for irrigation. It will withstand long periods of drought due to a very deep root system but is unproductive under such conditions. Alfalfa can tolerate both extremes of heat and cold. It is best adapted to deep loam soils with porous subsoils. Good drainage is essential and the plant requires a large amount of calcium for satisfactory growth. (Martin et al. 1975).

Botanically alfalfa is an herbaceous perennial legume that may live 15 to 20 years or longer in dry climates unless destroyed by insects or diseases. Red clover thrives in a cool, moist climate and makes its best growth on fertile, well-drained soils of pH. 6.6-7.6 that contains an abundance of lime.

Clover failure, the partial or complete loss of stands either in the seeding year or second year, may be caused by an unfavourable soil condition, winter injury, disease or insects. (Martin <u>et al.</u> 1975).

Wild red clover is extremely valuable, most of the plants being short-lived perennials. Forms exist that are early, late, smooth, hairy, prostrate, erect and semi-erect. (Martin et al. 1975).

Factors Influencing Brotein Content of Leaves

(a) Light and Temperature

Bathurst and Mitchell (1958) in controlled growth cabinet experiments on the effect of light and temperature on chemical composition of pasture plants found soluble nitrogen showed marked changes with temperature and light. Species studied included perennial ryegrass, (Lolium perenne), short rotation ryegrass (Lolium perenne x L. multiflorum), paspalum (Paspalum dilatatum), white clover (Trifolium repens), subterraneum clover (Trifolium subterraneum) and lotus major (Lotus uliginosus).

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With increasing temperatures $(45^{\circ}F. to 95^{\circ}F.)$, nitrate tended to increase and amino nitrogen to decrease. Nitrate was higher in plants grown in the shade at high temperatures. Amino acids differences were not as great but, generally, shade grown plants were higher in amino nitrogen than those grown in full light.

With advancing maturity and increasing light intensity, crude protein and in vitro digestibility of alfalfa decreases. (Garza <u>et al.</u> 1965). Forage composition and digestibility are influenced by many factors the most important of which are light, temperature, age of plant and water. Garza suggests that the higher content of crude protein at low temperatures is due to a slower maturity of leaves, less rapid hydrolysis of protein and slower translocation of nitrogen from the leaves. Low light intensity favours a high percentage crude protein and a slight increase in cellulose percentage but causes a decrease in dry matter and soluble carbohydrates. High temperatures causes a decrease in crude protein percentages and in vitro digestibility but an increase in cellulose and soluble carbohydrates. Crude protein and in vitro digestibility decreased markedly with maturity while percentage cellulose increased.

Reduction of light intensity with increasing temperatures progressively increases nitrate content, as reported by George (1967). Nitrate, over a period, continued to increase at low light intensities whereas it decreased at high light intensities. Deinum (1966) stated that the dry matter production as well as the dry matter percentage increased with higher light intensities. Crude protein, crude fiber, ash and NO₃ were lower at the higher light intensities. At a higher temperature, the dry matter yield and dry matter percentage were higher and contents of crude protein, ash and water soluble carbohydrates were lower.

Another study by Deinum and Dirven (1974) indicated that higher temperatures always cause a lower digestibility of herbage. Higher light intensities cause a depression in crude protein and crude fiber but show little effect on digestibility.

(b) Other Factors

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Arkcoll and Festenstein (1971) reported that the main factors influencing production are nitrogen fertilizer, age at harvest, seeding rate and climate. The highest amount of protein is usually obtained by harvesting just before vegetative growth ends and floral growth dominates. Both clovers and lucerne (alfalfa) regrow and yield well when cut at bud stage. They may be cut before this with little loss in yield. Cutting at an immature stage, however, results in slimy, wet pulp which is difficult to handle.

Chemical constituents in the herbage of forage species that are important in animal nutrition, including total digestible nutrients, sugars, protein, carotene, amino acids and mineral elements, decrease in concentration with advances in maturity (Smith 1975). The changes in concentration of chemical constituents can be more rapid in one species than another. For example, the changes usually are more rapid in alfalfa than in Ladino clover since the harvested product for Ladino clover is essentially all leaf tissue.

Loganathan and Krishnamoorthy reported that lucerne cuttings taken at thirty day intervals provided maximum yield of lush vegetation and leaf protein concentrate. Deinum (1966) reported that dry matter production decreased while dry matter content is increased by water shortage. The contents of crude protein and ash increases with drought.

Water shortages also result in higher digestibility (Deinum and Dirven 1974).

Factors Influencing Extractability of Protein

Published tables, listing extractability of protein from the leaves of many species, can be very misleading because extractability depends on many factors other than species (Pirie 1978). Early experience justified the tentative generalization that the greater percentage of protein in its dry matter the greater the percentage of that protein that will be extractable from a given species by a given technique. An example was the observation that the yield of leaf protein per m^2 was smaller from a plot of kale (Brassica oleracea) given phosphorous and potassium than from an unfertilized plot because, althought the dry matter yield was increased by 68%, the amount of nitrogen in the dry matter was diminished by 56%. The generalization holds only for comparisons between different treatments given to plots of the same species; species containing more protein do not necessarily extract better than others. It does, however, follow that there is a disproportionate advantage in harvesting leaves that are young, well manured and well watered. They will not only contain more protein but more protein will be extractable. Species also differ in the extent to

which maturity diminishes extractability.

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Pirie (1978) also reports that the ratio of protein nitrogen differs between species and within species depending on cropping practices, temperature and possibly the time of day at which the leaf is cut. Nonprotein nitrogen is more difficult to use in commercial practice than nitrogen in leaf protein, or associated with the fibre residue. Species and conditions should be chosen in which non-protein nitrogen is as small a fraction of the total nitrogen as possible. Some species produce extracts which are glutenous e.g. comfrey (<u>Symphytum asperrimum and</u> <u>Symphytum officinale</u>), or sweet potato (<u>Ipomoea batatas</u>) or forms an intractable mass of froth (e.g. some varities of lucerne).

Yield of leaf protein concentrate is dependent on temperature, dry matter, fiber and protein content of alfalfa. Yield of juice is also dependent on alfalfa fiber and dry matter content (Edwards <u>et al</u>. 1978).

Pirie (1978) also indicates the general phenomenon of the decline in yield of extractable protein at about the time of flowering.

Machinery Used in Fractionation

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Once harvested, the fresh material must be processed to separate the protein containing juice from the fiber.

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Many designs of screw-expellers and sugar cane rolls have been tested at Rothamstead but all have been found unsatisfactory. Screw-expellers were rejected because they consumed a great deal of power and because the continued rubbing in them introduced an undesirable amount of finely divided fiber into the juice. For reasons of both economy and to avoid over-heating of the material in the machine, power consumption must be kept low. Rollers were also found to be undesirable as several passes of material were required to get satisfactory liberation of juice. (Pirie 1971).

Researchers decided that pulping and pressing could probably not be managed on a large scale in one unit. As a result, a series of pulpers able to handle one ton of crop per hour were designed. In a conventional fixed-hammer mill, the plant material stays inside until it is sufficiently ground to pass through a grid. Pulp from a crop containing 80-93% water forms a mass, clogging the grid. Clogging can be overcome either by drying the material so that it will blow through or wetting it so it will flow through. Drying coagulates the protein on the fiber and wetting dilutes the resulting extract. (Pirie 1971). One early (1970) model of pulper had a cylinder within which a set of beaters, fixed to an axial shaft, rotated. For very tough crops, some prongs could be inserted through the casing into the spaces between the beaters so as to break the flow and increase the amount of pulping. The crop was fed in one end of the pulper and the pulp discharged at the other. It came out whether it had been properly pulped or not and it was, therefore, almost impossible to choke the pulper. Control over the amount of disintegration was given by using beaters differing slightly in form, by the use of prongs and by varying the speed. (Pirie 1971).

Much of the protein in extracts from pulped leaves is in chloroplast fragments and other particles that are visible under the microscope. If pressure is applied to the pulp in such a manner that a thick compacted fiber layer is formed, these particles are filtered off. The basic principles of press design are therefore: the pressed layer should not be more than 6 mm. thick, pressure should not be applied suddenly, and the pressure should be maintained for several seconds so as to allow time for the juice to run away. Pressure of 2 kg./cm² is sufficient to press out 90% of the juice that is extractable at very much greater pressures. (Pirie 1971).

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One satisfactory press, from Rothamstead, has an endless belt of woven nylon coated with PVC, tensioned and passed around a pulley with a cylindrical face of perforated metal. The pulp flies out from the pulper onto the inner face of the belt so that it is pressed between the belt and the perforated face. The juice is pressed through the perforations into the pulley and then runs out over its edges into a tray. An auger removes the pressed fiber from the exposed surface. A small amount of the fiber is pressed through the perforations and sometimes some is pressed out sideways. This fiber may be removed by straining the juice.

Products Of The Fractionation Process

Kohler and Knuckles (1977) reported that when alfalfa is ground and pressed the juice contains 35-60% of the crude protein but only about one third of the solids of the raw material. The remaining two thirds is in the form of a pressed pulp and must be effectively utilized as a feed if protein recovery from the juice is to be economically feasible.

(a) Juice

(1) Preservation Methods

Leaf juice may be stored whole for short periods of time but rapidly putrefies and, therefore, must be preserved unless quickly fed. (Tilley and Raymond 1957). Processing generally involves heating to precipitate a green curd containing about 15% of the original dry matter. The remaining dry matter is lost in the supernatant liquid usually referred to as deproteinized juice (Dumont and Boyce 1976).

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Pirie (1975A) states that heat coagulation is generally accepted as the most satisfactory method of making protein curd. The extracted "whey" is returned to the land where the N. P. and K. would be useful and the 1 - 3% carbohydrate used as a soil conditioner.

Cheeseman (1976) observed variations in the composition of whole and deprotenized juice and attributed this to seasonal effect and amount of applied fertilizer. Freshly expressed juice undergoes fairly rapid deterioration involving a drop in pH and increase in non-protein nitrogen both of which are related to storage temperature. Heat treatments inactivate endogenous enzymes but further treatment is necessary to control microbial spoilage. Several means of preservation were tried such as heat (steam injection to raise temperature to 85° C.), pH 3.0 and 4.5 (by addition of concentrated hydrochloric acid, formalin (0.1 and 0.2% V/V) and sodium metabisulphite. It was concluded that adjustment of the pH to 3.0 \pm 0.2 together with added sodium metabisulphite would satisfactorily preserve heat-treated forage juice for a period of several weeks at ambient temperatures.

A similar procedure was described by Braude (1974). Tests showed essential amino acids remained constant during storage but, after six months, a slight loss of phenylaline and leucine was evident.

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Wisconsin workers (Anonymous 1977B) carried out experiments on separation and preservation of plant juice by anaerobic fermentation with only bacteria normally found on leaves and stems carried into the juice as innoculum.

Before the Wisconsin work using fermentation is further. reviewed, organisms and processes involved in silage production should be reviewed.

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During the fermentation process of silage, lactobacteria from leaf material multiply using the soluble carbohydrates of the material producing organic acids (mainly lactic and acetic acid), thus lowering the pH of the material. Also contained on the leaf material is the undesirable bacteria clostridia which breaks down the organic acids and sugars to produce butyric acid. These bacteria are also responsible for the breakdown of proteins in the material. Once clostridia start to multiply, they use up organic acids and sugars, reducing the amount of acid being produced and the pH increases. Decomposition of the protein produces ammonia which neutralizes some of the lactic acid which further increases the pH. As a result, the juice becomes unstable and decomposition continues. If material is over 82% moisture, pH must be 4.0 or lower to prevent decomposition. Immediate addition of organic acids reduces the pH of the material and this encourages the bacteria that ferment the sugars producing lactic acid. As a result of lactic acid production, there is a further steady fall in the pH. In practice, the pH reached may be no lower than that reached by a normal fermentation process without additives, but silage with additives appears to be more stable during long-term storage. For crops which are naturally low in sugar, acid fermentation can be improved by the addition of molasses. Increased availability of sugars allows more organic acids to be formed, aiding in storage (Anonymous 1977A).

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Wisconsin workers report plant juice has a pH from 5.5 to 6.0 when first expressed but, when air is excluded, acid-forming bacteria multiply forming organic acids and carbon dioxide, as occurs in silage production. The pH drops within one to four days and, at this point, almost all of the protein precipitates as a fine coagulum which slowly settles out. Fermented samples have been maintained at room temperature for periods from a few weeks to four years without spoilage. Spoilage may occur after a period and is apparent by a gradual increase in pH. This spoilage may be due to a slow leakage of air into the containers or a change in type of fermentation. (Anonymous 1977B).

Fermentation of alfalfa juice, low in fermentable carbohydrates, was most subject to spoilage and this was reduced by the addition of molasses which is converted to organic acids in the fermentation process. (Anonymous 1977B).

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Protein yielded by fermentation of alfalfa juice was only two-thirds of that by heating. Alfalfa juice is high in proteolytic enzymes which converted some insoluble protein to soluble peptides or amino acids in a six day fermentation. Shorter fermentation times have given higher yields from alfalfa, up to 88% of that from heating. (Anonymous 1977B).

Fermentation of cassava juice on the other hand yielded 51% more than by heat coagulation. It thus appears that in some cases coagulation by anaerobic fermentation may increase the yield of plant juice protein by formation of single cell protein from carbohydrates and non-protein nitrogen in the juice. In other cases, proteolytic hydrolysis of plant protein may decrease yields. (Anonymous 1977B).

It is felt that coagulation of plant juice protein by the anaerobic fermentation process can result in significant energy savings when compared to heat coagulation. (Anonymous 1977B).

Collection of fermented coagulate within two weeks of the onset of fermentation is recommended with the deproteinized juice used as a fertilizer. Moist coagulum can then be incorporated into feeds. (Anonymous 1977B). Arkcoll (1973) found that 2% acetic acid added to the wet leaf protein was adequate to prevent growth of HCl tolerant fungi. Acetic acid is more effective than lactic acid and salt both of which must be added at 15% to completely inhibit microbeal growth. It has also been found that the addition of 3.5% W/W formic acid helps prevent spoilage. Subba Rau <u>et al.</u> (1967) also reported that 2% acetic acid in wet cakes preserved them satisfactorily.

Dry protein can be handled and transported more conveniently and cheaply than wet material. As long as the protein is kept below 9% moisture, xerophilic fungi are unable to grow. When drying protein material, care must be taken to prevent a black gritty material on grinding. Oxidation of lipids in the product may be a problem and can be avoided by extracting these with a solvent such as acetone. (Arkcoll 1973).

(2) Feeding Value

Proteins are built from twenty amino acids eight of which must be supplied in the diet of non-ruminant animals. The limiting amino acids in most plant proteins, including forages and plant juice protein, are the sulfur amino acids methionine and cystine. These sulfur amino acids may be oxidized in the presence of air by oxidative enzymes in the juice. Data suggests that anaerobic fermentation may give concentrates of better nutritive value than that coagulated by heat. Saponins in alfalfa juice are destroyed in the fermentation so that the fermented juice or protein concentration is less toxic. (Anonymous 1977B).

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Oshima and Oouchi (1976) fed rats a diet containing Ladino clover leaf protein concentrate as a sole protein source to supply 10% crude protein. It was found that the first, second and third limiting amino acids of the Ladino clover leaf protein concentrate for growing rats were methionine, lysine and threonine, respectively. The biological value of the protein concentrate was 21 and was improved to about 90 by supplementing the three limiting amino acids. True digestibility of protein in the protein concentrate was slightly lower than 80%.

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Alfalfa protein concentrate was also tested as a protein supplement in rat diets by Myer & Cheeke (1975). It was found that once lysine and methionine deficiencies were corrected it was equivalent to soybean meal as a protein supplement.

Fermented whole alfalfa juice and fermented moist coagulum have been, incorporated into swine rations. Pigs fed the whole fermented alfalfa juice which had been stored for a long period of time grew to dislike it. This could have been due to changes in the juice or excessive mineral intake from the juice. (Anonymous 1977B).

If pigs are fed fresh unpreserved juice, consumption is good but over-all performance, especially the feed-gain ratio, is lower than mealfed animals. (Mitchell 1978).

n N Braude (1974), indicated that true protein nitrogen in lucerne juice could have considerable value for growing pigs. Care must be taken in handling juice as substantial portions of true protein nitrogen may be lost by inadequate processing of juice during storage. No difficulties were experienced in feeding lucerne juice to young pigs but it was suggested that it may not be fed to pigs until they reach 25-30 kg. For optiumum feeding results, juice should be fed on the basis of true protein value rather than on its total nitrogen value. This indicates the pig is unable to make significant use of the non-protein nitrogen fraction. In order to achieve performances comparable to those of all meal-fed pigs, juice should replace only one-half the normal protein supplement up to 54 kg. liveweight and all of it thereafter.

Braude <u>et al.</u> (1976) concluded that either grass or lucerne juice, fed fresh or preserved, can supply a substantial amount of protein in the diets of growing pigs. Problems that arise from feeding the juice fresh, such as bulkiness and palatability, may restrict full replacement of either fish or soybean meal in diets.

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(3) Deproteinized Juice

Deproteinized alfalfa juice constitues over one-half of the weight of the harvested green alfalfa and must be disposed of. It contains relatively large amounts of N and K as well as smaller amounts of P, Ca, Mg, and microelements and is, therefore, useful as a fertilizer. When deproteinized alfalfa juice was applied at depths greater than 1.25 cm., plant damage occurs. Per hectare yields of crude protein were increased by fertilization with deproteinized alfalfa juice. Available P and exchangeable K in the soil generally increased and pH was maintained at satisfactory levels as a result of Ca and Mg additions from the juice. (Ream et al. 1977).

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Walgenbach <u>et al.</u> (1977) concluded that unknown phytotoxins contained in the deproteinized juice or produced as microflora breakdown products were responsible for the plant damage at high application rates.

(b) Pulp

The composition and nutritive value of the pressed crop depends on the composition and nutritive value of the whole crop from which it is derived and the machines used to express the juice. The pressed crop contains about 50-80% of the dry matter harvested in the whole crop and, therefore, the utilization of this fraction is important. Pressed crops can be fed fresh, ensiled or artificially dried, to ruminants. (Connell & Houseman 1976). Fractionation reduces crude protein by up to 25%, digestible organic matter values by about 5% and total ash content by almost 35%. In addition, the pressed crops have been pulverized during the process. (Connell & Houseman 1976).

Promising results indicate that fresh pressed crops can be utilized effectively by cattle. Pressed crops ensile easily although difficulties have been reported with silages made from pressed lucerne. This is because of the relatively lower soluble carbohydrate content of the lucerne which can be overcome by the addition of molasses or acid. Results from feeding trials indicate that artificially dried pressed crops can be reliably allocated to cattle on a basis of crude protein or digestible organic matter or metabolisable energy values. (Connell & Houseman 1976).

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MATERIALS AND METHODS

Field Area

The Nova Scotia Agricultural College field plot area is situated near the Training School on the Brookside Road in Bible Hill near Truro, N. S. The area is a gently sloping field of soil classified as a Truro sandy loam, which was derived from red sandstone. The soil is fairly well drained by a recently installed subsurface drainage system. The pH of uncultivated soil of this type is recorded as A_0 horizon = 4.2, $A_2 = 4.6$, $B_1 = 4.6$, $B_2 = 5.0$ and C = 4.6.

Forage Material

Ladino clover (no specific cultivar) material for experiments was grown on plot 31-3. This plot had lime applied at the rate of 1 t/ha. in the fall of 1975. Actual pH of the area was 6.7. Area was seeded May 27, 1980. Six hundred kg./ha. 5-20-20 + 2B was added at seeding. Fertilizer was applied to plots at the rate of 600 kg./ha. 5-10-30 in early spring and 200 kg./ha. 0-0-60 after each cut.

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Ottawa red clover was obtained from the double cut red clover trial plots (area 22-2) which were planted in 1980 and treated with the same fertilizer treatments as the Ladino. Similarly, Iroquois alfalfa was obtained from the regional alfalfa trial (plot 22-1) also planted in 1980. pH of both areas was 5.9.

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Equipment and Machinery

The forage harvester used in all cases was a modified Haban flail type mower.

The pulper and press used in the fractionation process was a Weedon mini pulper and press. The manufacturer's specifications are as follows:

A free standing machine basically of rolled hollow section steel construction. One side is removable for belt servicing.

At one end of the frame is a solid roller, zinc plated, running in plummer blocks which in turn are mounted on a carriage. This carriage is spring loaded by four springs of $\frac{1}{2}$ tonne/inch (500 kg./25mm.) rate, the tension of which is adjustable. This carriage in turn is part of and integral with a gearbox of 1400/1 ratio and motor, fixed to the sliding

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carriage via rollers and rails mounted to the base of the main frame so that with variation in the centres of the drive roller the whole unit can move to provide the needed adjustment automatically.

At the other end of the frame is a perforated roller running in two fixed plummer blocks. Connecting both rollers is a cotton belt, PVC covered food grade, with guide rollers. A scraper is positioned just to the rear of the perforated drum to scrape off residue from the belt, and immediately beneath the scraper is an auger in contact with the perforated drum to remove the dry fodder. The auger is driven by motor and gearbox, and is mounted at 90° to the main axis of the machine.

A chute is provided to guide the product from the mini pulper onto the belt in front of the perforated drum. A stainless steel tray with drain is positioned under the drum.

Direct on line starters are provided for both motors. Weight is approximately 1650 lbs. (750 kgs.).

Approximate dimensions of the Press:

55" (1396 mm.) high 24" (609 mm.) wide 90" (2284 mm.) long



Figure No. 2

Weedon mini pulper and press illustrating total machine top chute for loading forage

Figure No. 3

End view showing perforated TA drum, belt and conjecting pan for juice



Rear view showing collecting chute for pulped material and auger for removing pressed cake



Protein Analysis (Kjeldahl Method)

<u>Digestion</u>: One gram samples of dried material were weighed into large digestion tubes. Two Kjeltabs M/3.5 ($3.5g.K_2SO_4$ plus 175 mg. H_2O) were added to each tube. Fifteen ml. digestion acid ($1OO_2$ parts concentrated H_2SO_4 plus 5 parts concentrated H_3PO_4) were added as well as 5 ml. of 35% hydrogen peroxide. Samples were digested at $420^{\circ}C$. for fifteen minutes in a Kjeltic System I. After digestion, samples were cooled 5-10 minutes and diluted with 75 ml. distilled water.

<u>Distillation:</u> Twenty-five ml. 4% Boric Acid solution with mixed indicators were poured into 250 ml. flasks labeled corresponding to the digestion tubes. Ten ml. sodium thio sulphate solution $(Na_2S_2O_3 \times 5H_2O_3OOg_1)$. water) were added to the digestion tubes. Tubes and corresponding prepared flask were then placed into the Kjeltic System II. Fifteen ml. of sodium hydroxide (40%) was dispensed into the tube. Distillation time was 3-4 minutes. Samples were then removed. Contents of the tube were discarded and contents of the flasks titrated with hydrochloric acid.

<u>Calculations</u>: The percentage protein of the samples was calculated using the following formula:

Percentage Protein = $\frac{(ml. HCl - blank) \times normality \times 14.007 \times f \times 100}{(mg. sample)}$ In this case, f = 6.25.

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Statistical Analysis

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Production of Protein Concentrate:

(a) Ladino Clover

Randomized complete block design was used for analysis of total and of individual cuts for dry matter yield, fresh juice yield, protein concentrate yield, percent protein of protein concentrate and actual protein yield.

A combined analysis for each of the above factors was carried out using the split plot design. Treatments, means being first cut dates, were among the main plot units and cut means were within main plot units.

(b) Other Species

Separate analysis using randomized complete block design was used to compare means of total dry matter yield, juice yield, protein concentrate yield, percentage protein in protein concentrate and actual protein yield from the first cut and the total seasonal yield of red clover, Ladino clover and alfalfa, all first cut at the 1/10 bloom stage.

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Analysis of total yield of protein concentrate, percent protein of protein concentrate and actual yield of protein using various storage methods was carried out using the split plot design. Storage treatments were among the main plot units, and time within main plot units.

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Production of Protein Concentrate

(a) Ladino clover - A randomized complete block design with six treatment (first cut dates) was set up. First cut treatments began June 11, 1981, and continued weekly for six weeks. Growth stages at each first cut were identified. Material was recut at 28 day intervals. Harvest area was $.8m \times 4.5m (3.6m.^2)$. The yield and dry matter of fresh cuttings were recorded. Dry matter was determined by weighing a small random sample of forage in a paper bag, drying in the dryer for twenty-four hours, then weighing dry material.

Table No. 1Growth stages used in fractionation experiment at firstcut dates of the Ladino clover

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First Cut Date	Growth Stage
June 11	Vegetative - Pre-bloom
June 18	Bud
June 25	Early bloom (1/10)
July 2	Full bloom
July 9	Full bloom
July 16	Late bloom

Fresh cut material was processed through the Weedon mini pulper and press. Yield and dry matter of both the pulp and juice fractions were recorded. The pulp was disposed.

Fresh juice from each plot was collected in a separate 41. glass jar, tightly covered and placed in a cool, poorly lit room one week to ferment. After the one week fermentation period, yield of protein concentrate and deproteinized juice were recorded. Dry matter percentage and percentage - protein of the protein concentrate were determined. Total yields of cuttings, protein concentrate and actual protein were calculated in kg./ha.

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(b) <u>Other species</u> - Iroquois alfalfa and Ottawa red clover were harvested at 1/10 bloom and processed as was the Ladino. The second cut of Ottawa red clover was taken at full bloom fifty-five days after first cut. Alfalfa's regrowth period was forty-five days and it was also at full bloom stage. Calculations similar to those with Ladino were made and species were compared.

Juice Preservation and Storage

(a) In 1980, a non-replicated trial with six treatments and six first cut dates was carried out. Fresh juice was placed in 1 1. pyrex flasks, tightly covered and treated as follows:

1. Control - no treatment

2. 3.5% volume/volume acetic acid added immediately

3. As #2, but added at one week

4. 10% volume/volume molasses.

5. As #2, but deproteinized juice removed at one week

6. Heat added to precipitate protein.

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Yield of protein concentrate was recorded and percentage dry matter and percentage protein were determined. Spoilage was also rated.

(b) In 1981, a 2 x 13 m. area of Ladino clover was harvested with the Haban flail-type mower on June 9th, 23rd and July 7th, each date being a first cut.

Material was processed through the Weedon mini pulper and press. The resulting juice was divided into 16 l l. pyrex flasks. Flasks were then randomly assigned to one of the four following treatments:

- 1. 3.5% volume/volume acetic acid added immediately
- 2. As 1, but added at one week after protein had coagulated due to fermentation process
- 3. 10% volume/volume molasses
- 4. Control no treatment.

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Spoilage was monitored by pH measurements at the beginning of the treatments, at one week and one month later. At one month, all deproteinized juice was discarded and yield of the protein concentrate recorded. Dry matter percentage and percentage protein of the protein concentrate were determined. Protein concentrate material from treatments 1 and 3 were saved for use in rat experiments.

RESULTS AND DISCUSSION

General Procedures Used in Reporting Data

The yields of the separate fractions of Ladino clover are tabulated showing yields of cut 1, cut 2, cut 3, and total yield.

Yield totals for red clover and alfalfa aré based on two cuts per season.

Analysis of variance tables corresponding to tables of results are found in the appendix.

Production of Protein Concentrate

(a) Ladino clover

Table No. 2 Dry matter yields of Ladino clover forage material (kg./ha.) cut 1, cut 2, cut 3 and total yield

First cut date	Cut 1	Cut 2	Cut 3	Total
July 9	3502a	1447ab	1202ab	6150a
July 2	3506a 4	1386ab	1953bc	5945a
June 25	3158a	1 651 a	995cd	5804ab
July 16	3506a	1183b	815d	5504ab
June 18	2127b	1634a	1336a	5097ъ
June 11	1733b	1433ab	950cd	4115c
Mean	2922	1456	1059	5436

Means within each column with common letters are not significantly different at the .05 level

See appendix tables No. 1 - 5.

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Examination of the dry matter yield, results of the ladino production experiment (Table No. 2) indicates yield of fresh cut material is greater with delayed first cutting date. This is expected (Smith 1975) as there is a natural accumulation of material with time. Total yields of fresh cut material appear to be significantly less when material is harvested at the pre-bloom stage.

Plots yielding largest amounts of dry matter with the first cut tended to yield less on the second cut. Cut one generally yielded more forage material than cut two which, in turn, yielded more than cut three. The results also indicate yields from the second and third cut material, all cut at 28 days regrowth, vary somewhat. This could possibly be due to weather variations both before and after the cuts or to the effect of previous cutting treatment.

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Figure No. 5 indicates a levelling off of dry matter production with the first cut after material reaches full bloom.



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Fresh cut material processed through the pulper and press results in both a juice and pulp fraction Table No. 3 indicates fresh juice yields on a dry matter basis.

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Table No. 3 Dry matter yield of fresh juice (kg./ha.) of Ladino clover from cut 1, cut 2, and cut 3, forage material and total

First cut date	Cut 1	Cut 2	<u>Cut 3</u>	Total
June 18	361a	303a .	206a	870a
July 2	369a	, 229cd	200a	798ab
June 25	· 361a	247bc	172ab	780ab
June 11.	321a	266b	182ab	77 4ab
July 9	289a	233cd	225a	7 46b
July 16	264a	212d	118b	593c
Mean	328	248	184	760

Means within each column with similar letters are not significantly different at .05 level. g_{π}

See appendix Table Nos. 6 to 10.

There appears to be no significant differences in dry matter yield of juice with later first cut dates of forage material. Yield of juice from second cut material is less than that from first cut material. Third cut material, in turn, yields less dry matter from juice than second cut material.

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Earlier second cut material appears to yield more dry matter from juice than material with a second cut taken later in the season. Total yields of dry matter from fresh juice obtained throughout the season are significantly different. Lower yields are obtained with delayed first cut dates, especially after full bloom has been reached.

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The percentage of dry matter yield (weight) of fresh juice from the fresh forage material processed to produce that juice was calculated and is tabulated as follows:

Table No. 4 Dry matter yield of fresh juice expressed as a percentage of the fresh Ladino clover processed to produce the juice

		Percentage	
First cut date	<u>Cut 1</u>	Cut 2	<u>Cut 3</u>
June 11	21.29	15.35	19.16
June 18	16.97	18.54	15.42
June 25	11.43	. 14.96	17.29
July 2	10.52	16.52	18.99
July 9	8.25	16.1 0	18.72
July 16	7.53	17.92	14.48

A decline in percentage of dry matter yield of fresh juice from fresh forage to produce the juice can be seen with an increase in age of material in cut 1. Older forage material is higher in dry matter content therefore juicing less easily, thus explaining the tabulated decline in juice extracted from older material.

Although early cut plots yield less forage material than later cut plots, the early cut material yields a higher proportion of juice. Even though no significant differences in dry matter yield of fresh juice can be seen with first cut dates delayed, cutting of pre-bloom material is recommended as less forage material needs to be cut and processed to yield an equivalent amount of juice. Processing of early cut material would not only save energy and work but increase juice yield per hours of machine operation.

The percentage of dry matter of fresh juice from fresh cut forage material to produce that juice for second and third cuts are also shown in Table No. 4. All material is the same age but great variations are evident.

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Figure No. 6 illustrates the contribution to total juice yield of juice from each of the three cuts. First cut material harvested before full bloom appears to yield more juice.

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If fresh juice is not used immediately, some form of treatment must be undertaken for material to be stored and prevent spoilage. Fermentation of fresh juice is one such method of storage and results in a protein concentrate and deproteinized juice. The fermentation process also concentrates the volume.of protein material.

Table No. 5 Dry matter yield of protein concentrate (kg./ha.) from 3 cuts

First cut dates	<u>Cut 1</u>	Cut 2	, <u>Cut 3</u>	Total
June 18	183a	148a	100a	430a
June 11	162a	127 [°] b	87a	376ъ
July 2	152ab	96de	116a	364Ъ
June 25	163a	83e	99a	345b
July 9	120bc	106cd	102a	328bc
July 16	99c	116bc	72a	287c
Mean	146	113	. 96	355

Means within each column with similar letters are not significantly different at .05 level.

See appendix tables Nos. 11 to 15.

of Ladino clover

Examination of Table No. 5, protein concentrate yield (dry matter basis, indicates greater yields of protein concentrate from juice expressed from earlier first cut material. This would be expected as younger material is generally higher in protein content, (Smith 1975), which decreases with increasing age of the material. The percentage of dry matter yield of protein concentrate from fresh juice needed to produce the concentrate may also be calculated as follows:

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Table No. 6	Dry matter yield of protein concentrate expressed as a
-	percentage of fresh Ladino juice required to produce the
,	concentrate

		Percentages	
First cut dates	. <u>Cut 1</u>	Cut 2	Cut 3
June 11	50.47	47 - 74	47.80
June 18	50.55	48.84	48.54
June 25	45.15	33.60	57.56
July 2	41.19	41.92	58.00
July 9	41.52	45.•49	45.33
July 16	37.50	54.72	61.02

Figures from cut 1 in the above table indicate that at least 50% of the weight of fresh juice at the first two or pre-bloom cutting dates is retained as protein concentrate after the fermentation process. Less protein concentrate is yielded per unit of fresh juice at later first cut dates.

These trends correspond with the findings of Arkcoll and Festenstein (1971) who reported that the highest amount of protein is usually obtained by harvesting just before vegetative growth ends and floral growth dominates. No flowering appeared before June 18th and growth stage at June 25th may best be described as 1/10 bloom stage.

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Actual dry matter yields of protein concentrate from second cut and the ratios of yield of concentrate per unit of fresh juice to produce the concentrate vary considerably.

Recommendations for the highest protein concentrate yield therefore would be to cut, fractionate and ferment material at the pre-bloom stage. Succeeding cuts should be taken at about one month intervals.

Water could possibly be added to forage material during the pulping procedure to aid in collection of protein from pulp but added volumes of water would necessitate larger containers for the fermentation process.

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More replicated trials, where water is added at time of pulping, are necessary to establish whether the addition of water indeed would result in greater yields of protein concentrate per unit of fresh forage material.

Figure No. 7 indicates the contribution to total protein concentrate yield from each of the three cutting dates.



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The actual percent protein of the protein concentrate as presented in Table No. 7 appears consistent throughout the first and second cuts, with the second cut being of higher value. Values of third cut material appear to vary greatly with percentage protein of third cut material being lower. The only explanation for these variations is a combination of environmental factors.

Table No. 7 Percentage protein of protein concentrate - Ladino clover

Dry matter basis

First cut date	<u>Cut 1</u>	<u>Cut 2</u>	~ · ~	eighted nean
July 16	37.96a	40.56a	42.18a	40 . 23a
July 9	38.08a	40.48a	38.68b	39.08ab
June 25	38. 75a	40.67a	36.98bc	38 .8 0ab
June 11	38. 49a	39.67a	37 . 7.96	38.65bc
June 18	38.97a	40 . 26a	33•71d	37.65Ъс
July 2	36.26a	40 . 47a	35.16cd	37•29c
Mean	38.09	40.35	37-42	38.62

Means within each column with common letters are not significantly different at the .05 level.

See appendix table Nos. 16 to 19.

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The actual protein yield in protein concentrate was calculated using the percent protein and yield of protein concentrate. These values are recorded in Table No. 8.

Table No. 8	Actual dry	matter yield	of protein in	protein conc	entrate
	(kg./ha.) -	Ladino clove	er		
First cut da		<u>Cut 1</u>	<u>Cut 2</u>	Cut 3	Total
June 18		7la	60a	3la	162a
June 11		62ab	50ab	33a	145ab
July 2		57ab	40cd	4la	138bc
June 25		63a	34d	37a	134bc
July 9	н 1	47bc	43bd	40a	130bc
July 16		38c	47bc	3 la	116c
Mean		56	46	36	138

Means within each column with common letters are not significantly different at the .05 level.

See appendix tables Nos. 20 to 24.

The earliest cutting dates for the first cut yielded the greatest amount of protein (dry matter basis). Trends were similar with cut number two but yields were less than in cut number one. Total dry matter yield of protein over the three cuts was greatest with the early (pre-bloom) first cut dates.

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Percentage of the actual protein yield in protein concentrate perweight of fresh juice to produce the concentrate through the fermentation process were calculated and are presented as follows:

Table No. 9Actual dry matter yield of protein in protein concentrateexpressed as a percentage of dry matter weight of freshjuice to produce the protein concentrate through thefermentation process

		Percentage	<u>e</u>	
First cut dates	<u>Cut 1</u>	' <u>Cut 2</u>	<u>Cut 3</u>	Total
June 11	19.31	18.80	18.13	18.73
June 18	19.67	19.80	15.05	18 . 62 [*]
June 25	17.45	13.77	21.51	17.18
July 2	15.45	17.47	20.50	17.29
July 9	16.26	18.45	17.78	17.43
July 16	10.44	22.17	26.27	19.56

Again, the declining values with increasing maturity of material at the first cut and the variation within the second and third cut material supports the recommendations already made.

If the values, total dry matter yield of protein in protein concentrate per total juice to produce the protein for one season, are examined, there appears to be very little variation.

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The lack of total variation may be due to the uncontrolled and unexplained variations found in the second and third cuts.

One final comparison to be made is that of the total protein in protein concentrate to the weight of fresh cut forage material processed to produce that protein.

Table No. 10 Total dry matter yield of protein in protein concentrate expressed as a percentage of total dry matter weight of

fresh cut forage material processed to produce the protein

First cut dates	Percentages
June 11	3.52
June 18	3.18
June 25	2.31
July 2	2.32
July 9	2.11
July 16	2.11

Above percentages support the recommendations in this report that pre-bloom material should be processed for best yields of protein with the least volume of forage material to be handled.

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Figure No. 8 indicates the contribution of actual protein from each cut to make up the total seasonal protein in protein concentrate yield.

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Figure No. 8 Actual dry matter yield of protein in protein concentrate (kg./ha.) from Ladino clover illustrating contribution from 3 cuts to total dry matter yield



b) Other Species

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Table No. 1!Comparison of yields of fractions of cut 1, Ladino clover,
red clover, and alfalfa (kg./ha. dry matter) cut at 1/10
bloom stage

<i>,</i>	Dry Matter <u>Yield</u>	Juice	Protein Concentrate	Percent Protein In Protein Concentrate	Actual Protein In Protein Concentrate
Ladiño clover	3158b	361Ъ	163b	38.75a	63a
Alfalfa	3478b	504a	216b	32.94b	71a
Red Clover	4614a	627a	308a	30.17b	93a
Mean	3750	497	229	33.95	76

Means within each column with similar letters are not significantly different at the .05 level.

See appendix tables Nos. 25 to 29.

When cut at the 1/10 bloom stage, red clover appears to yield significantly higher amounts of fresh forage material than either alfalfa or Ladino dlover. When the forage material is processed through the pulper and press, both red clover and alfalfa'yield significantly higher amounts of fresh juice than the Ladino. Red clover and alfalfa yield significantly higher amounts of protein concentrate which is lower in percent protein after juice is fermented. Because Ladino clover protein concentrate is significantly higher in percent protein, calculations of actual protein yield equal out so there appears to be no significant difference in protein yield among any of the three crops when cut at the 1/10 bloom stage. However, the actual value was much higher for red clover.

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Percentages as indicated in the following Table No., 12 have been calculated for fresh cut material of the three species studied.

Table No. 12Percentages of fractions red clover, alfalfa andLadino clover - cut 1

Species	Juice/Forage	Protein Concentrate/ Juice	Protein/Juice	Protein/Forage
Red Clover	13.58	49.16	14.84	2.02
Alfalfa	14.49	42.86	14.09	2.04
Ladino Clover	11.43	45.21	17.48	1.99

The fractionation process yields only between 11.0 and 14.5% of the weight of fresh cut forage material in the juice at the 1/10 bloom stage, with alfalfa giving the highest juice to forage ratio. Examination of the ratio of the protein concentrate to juice used to produce the concentrate indicates slightly higher yields of protein concentrate from the red clover juice than from the juice of the other two species.

The ratio protein yield in protein concentrate from juice to produce the protein, appears greater with Ladino clover but, because of the lower yield of juice per area, no differences in protein yield in protein concentrate per unit area can be detected.

If the total seasonal yields of fresh cut material are examined as in Table No. 13, red clover again leads in the production of forage material. However, there appears to be no differences in the total seasonal juice yield, protein concentrate yield, or yield of actual protein in protein concentrate.

Table No. 13Comparisons of total seasonal yields of fractions ofLadino clover, red clover and alfalfa (kg./ha. dry matter)based on 3 cuts/season of Ladino clover and 2 cuts/seasonof the other crops

Species	Dry matter 	Fresh juice	Protein ^C oncentrate	Actual protein in Protein concentrate
Alfalfa	6504b	711a	321a	106 <i>a</i>
Red lover	7784a	759a	389a	116a
Ladino	5804b	780a	346a	· 134a
Mean	6697	750	352	119

Means within each column with similar letters are not significantly different at .05 level.

See appendix tables Nos. 30 to 33.

Again, percentages of the various fractions have been calculated and are tabulated as follows:

Table No. 14 Percentages of fractions for red clover, alfalfa and Ladino clover - seasonal totals

Species	Juice to forage processed	Protein concentrate to juice processed	Protein in protein concentrate to juice processed	Protein in protein concentrate to forage processed	
Red clover	9•75	51.25	15.28	1.49	
Alfalfa	10•93	45.15	14.91	1.63	
Ladino clover	13.44	44.36	17.18	2.31	

These values appear to indicate that throughout the season more juice is yielded per unit measure of forage from Ladino. An explanation for this may be the fact that Ladino was harvested three times during the summer as compared to twice with the other crops. Ladino clover harvested at the second and third cuts would be more lush and, therefore, yield more juice.

An earlier cut of alfalfa and red clover would allow for a third cut. It can be speculated that if this regime were followed both red clover and alfalfa would produce more protein concentrate per area per season than. Ladino clover, although winter kill may be a major problem with this three cut per season treatment of red clover and alfalfa.

If red clover and alfalfa were cut at earlier and frequent stages, more protein concentrate may also be obtained per unit of forage material processed.

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Another reason may be the Ladino's growth habit, which allows the frequent harvest of material without harvesting the stems which is not as juicy as the leaf material. Lower yield of forage material per area of Ladino clover may decrease the significance of this.

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Total seasonal yield of protein concentrate per weight of juice to yield the concentrate appears higher in red clover, but the lower percent protein of the material lowers the actual protein in protein concentrate from juice ratio, to a point where there appears to be little difference, with the ratio of Ladino being slightly higher. The actual yield protein in protein concentrate per season from total seasonal forage material appears higher with Ladino clover.

It would seem from these tables that, on a seasonal basis, Ladino clover would not produce significantly different yields of protein per area and, in fact, less forage material and juice would need be processed to produce this protein. If protein production alone is the aim of a program, Ladino could be recommended as the crop to grow.

On the other hand, alfalfa and red clover yield more fresh forage material per area than the Ladino. Again, there are no significant differences in final protein yield per area between the three crops. Slightly more forage material needs to be processed to obtain the protein from alfalfa and red clover but there would also be more pulp produced. If the highest total feed production per area is the aim of the program, either alfalfa or red clover would be recommended. These crops produce more valuable pulp material, usable as ruminant feed, than Ladino and amounts of protein which are not significantly different. Because more forage material is required to be processed to produce red clover or alfalfa protein, more labour would be involved in handling greater volumes of forage material as well as extra energy used by machinery. Less protein per hour of pulper and press time could also be produced.

Choice of crops would depend on specific needs of the program as all species appear acceptable for use in a fractionation and fermentation process. Adaptation of the species to the area of production would also be a major factor in choosing the crop to be grown.

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Juice Preservation and Storage

(a) 1980

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Tables Nos. 15 and 16 show the protein concentrate and actual protein yields obtained in the 1980 non-replicated storage trial. The trend appears to be that highest yields are obtained when protein is coagulated immediately either by heat or lowering the pH as compared to treatments where fermentation causes protein coagulation. Greatest yields also appeared to be obtained at the earlier cutting dates.

Table No. 15 1980 storage trial - yield of protein concentrate (g.) per 100 g. of fresh juice

1								
 .	June_5	June 12	June 18	June 26	July 3	July 10	July 17	Mean
Control	373	3.30	4.21	4,152	2.07	2.97	2.32	3.30
Acid coagulation	5.78	4.60	5.41	6.72	2.58	3.91	3.91	4.70
Acid 1 week	3.92	3.65	6.64	5.08	2.25	2.81	2.25	3.80
10% molasses	4.17	2.88	4.96	5.76	2.84 .	4.12	3.92	4.09
Acid coagulated deproteinized juice removed	5•32	3.30	. 6.78	2.78	2.21	3.89	3.04	3.90
Heat	5.72	4.93	5.88	6.21	2.66	4.54	3•34	4.76
Mean	4.77	3.78	5465	5.18	2.44	3.71	3.13	4.09
Non morali oci	Fod		<i>(</i> .		0			

Date of harvest

Non replicated

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Table No. 16 1	able No. 16 1980 storage trial - actual protein Yield (g.) per 100 g.							
of fresh juice								
. <u>Date of harvest</u>								
	June 5	June 12	June 18	June 26	July 3	July 10	July 17	Mean
Control	1.14	1.16	1.24	1.48 [.]	.67	•96	•71	1.05
Acid coagulated	1.58	1.40	1.45	1.84	•79	1.12	1.10	1.33
Acid 1 week	1.17	1.16	1.37	1.51	•73	•94	•74	1.09
10% molasses	1.27	1.05	1.08	1.48	•70	•94	.83	1.05
Acid coagulated deprotfinized juice removed	1.69	1.23	1.76	1.07	.72	1.29	. 85	1.23
Heat	1.78	1.62	1.76	1.03	•91	1.71	1.08	1.41
Mean	1.27	1.27	1.44	1.40	•75	1.16	-89	1.19

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Table No. 17 shows the storage rating of the materials after approximately one month. Heat alone did not appear useful in prolonging storage. Use of acid and molasses appeared helpful in reducing spoilage.

Table No. 17 Ratings of 1980 stored material on the basis of smell

	Treatment	Storage rating
1.	Control	Spoiled
2.	Acid coagulation	Preserved
3.	Acid at one week	Preserved
4.	10% molasses	Preserved
5.	As No. 2 - deproteinized juice removed	Preserved
6.	Heat	Spoiled

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(b) 1981

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Table No.18 shows the dry matter yield of protein concentrate from 1981 storage trial.

Table No.18 I	<u>Dry matter yi</u>	eld of protein	concentrate us	sing various storage
Ĩ	methods (g./]	00g. fresh jui	ce) – Ladino ¢l	Lover 1981
Treatment	June 9	June 23	July 7	Mean
Acid ^c oagulation	4.41a	2.75a	3.02a	3.39a
10% molasses	3.20b	1.97b	2.58a	2.59b
Acid at 1 week	3.28b	1.33c	2.53a	2.38b
Control	2.60b	1.74bc	1.41b	1.92c
Mean	3.37	1.95	2.39	2.57
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Means within each column with similar letters are not significantly different at .05 level.

See appendix table No. 34.

Results of tests confirm suspicions that immediate coagulation of protein results in greatest yield of protein concentrate. Data also indicates that greatest yields are obtained when material is cut at earlier growth stages.

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The percent protein in the protein concentrate (Table No. 19) varies with treatments and dates. The mean values of three cuts indicate significantly higher percent protein of the concentrate when no treatment is applied. Lowest percent protein is observed where 10% molasses is added to fresh juice to aid the fermentation process.

Table No. 19 Percentage protein in protein concentrate using various storage methods - Ladino clover - 1981

Treatment	June 9	June 23	July 7	Mean
, Control	36.70a	39.56a .	35 . 15a	37.14a
Acid at 1 Week	30.76ъ	36.24b ,	33.24ab	33.41b
Acid Coagulation	34 . 86a	32.56c ·	30.22b	32.54b
10% Molasses	30.26ъ	31.150	25.46c	29.29c
Mean	33.15	34.88	31.27	33.10

Means within each column with similar letters are not significantly different at .05 level.

See appendix table No. 35.

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An explanation may be that the addition of the molasses and acetic acid dilutes the values making the untreated material appear higher in percent protein. This is supported by the fact that equal amounts of acetic acid were added at different timing and the percent protein values are not significantly different. When a higher volume of molasses was added, the percent protein value dropped. Q.

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Actual protein yield as shown in Table No. 20 is significantly higher with immediate coagulation of the protein with acid and also at earliest cutting date.

Table No. 20	Dry matter yield of actual protein in protein concentrate
	using various storage methods (g./100g. fresh juice) -
	Ladino clover - 1981

Treatment	June 9	June 23	July 7	Mean
Acid Coagulation	1.54a	•90a	•92a	1 . 12a
Acid at 1 week	1.00b	•48c	. 84a	•77b
10% molasses	•97ъ	.61bc	.68b	•75b
Control	•95b	-69b	•50c	•71b
Mean	1.12	.67	•74	•84

Means within each column with similar letters are not significantly different at .05 level.

See appendix table No. 36.

Table No. 21 shows pH of juice at the beginning and end of the storage trial. Both the acid and molasses treatments appeared to store well as evidenced by low pH.

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experimental dates in 1981 storage trial June 9 June 23 July 7 Initial Initial 1 Month 1 Month Initial 1 Month Acid coagulated 3.2 3.6 3.7 3.7 3.6 3.7 Acid at 1 week 5.8 3.7 5.5 3.6 3.5 5.3 10% molasses 5.6 5.1 3.6 3.8 5.3 3.7 5.2 Control - 5.8 5.6 5.3 5.5 5.8

Table No. 21 Initial and ending pH of stored material with three

The pH of the control, where no treatment was applied, was above 5.0 at one month in each of the three tests. As discussed in the literature review by Anonymous (1977A) silage material which is over 82% moisture must be kept at a pH below 4.0 to prevent spoilage. The high pH of juice could indicate activity of bacteria of genus Clostridium which are known to break down proteins as well as organic acids and sugar thus explaining the lower yield of protein as shown in Table No. 20 (actual protein yield) where no treatment was applied.

Material treated with acid immediately, or at one week, and with molasses, all showed pH readings of below 4.0 at one month. This would indicate the material was storing well.

Treatment with acetic acid immediately after juice expression causes an immediate drop of pH and protein coagulation. No time at all would be allowed for any clostridia activity and there would be no breakdown of protein. This would explain the significantly higher yields of protein

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Juice which had (1) acetic acid added at one week, after fermentation had begun and (2) molasses added immediately to fresh juice, both stored well but yielded less protein than where immediate coagulation of the protein occurred. During the slow process of fermentation, some protein breakdown may have occurred thus explaining lower yields of protein.

Above results indicate treatment of fresh Ladino juice is necessary for long-term storage of the material. Assuming palatability of material coagulated with acetic acid is not decreased, this method of protein concentration and storage could be recommended, as highest yields of protein concentrate are obtained and material stores well over a period of time. If palatability of material appears to be a problem, addition of molasses to the fresh juice before fermentation should be considered. Although there is a slightly reduced yield of protein with this method, long-term storage is possible.

Addition of the acetic acid after allowing natural fermentation for one week, although effective in prolonging storage, would not be recommended as the acid would be more wisely used immediately for reasons of increased protein yield and reducing the chance that rapid decomposition may occur causing spoilage before the acid could be added.

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Above results are comparable to trends reported by Wisconsin workers (Anonymous 1977B) working with alfalfa and results reported by Arkcoll (1973) who used acetic acid to prolong storage.

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CONCLUSIONS

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Although some of the results obtained in this report indicate interesting trends, much more work is necessary before meaningful conclusions can be drawn. This study, itself, raises more questions, and opens more doors for investigation than it answers.

The Weedon mini pulper and press used in the fractionation process, does not appear to be an efficient machine for extracting protein-rich juice from forage material. Equipment should be adjusted or redesigned so as to get more and better quality protein juice from the fresh forage. Time required to process material through this equipment is also excessive.

As determined in this study, harvesting and processing of prebloom Ladino Clover material yields the greatest amount of protein concentrate through the fermentation process. A further intensive study should be undertaken to see if these trends hold true with other forage legumes including alfalfa, birdsfoot trefoil and red clover.

Unexplainable differences in yield of juice and protein concentrate, percent protein in concentrate and actual protein in protein concentrate with different cutting dates of material of the same age (at second and third cut) were observed. From the results of these experiments, early and frequent cutting of Ladino clover material is suggested for greatest yields of protein concentrate with least amount of forage processed.

When cut at 1/10 bloom, no significant differences in first cut yield, or total seasonal yield of protein concentrate per area harvested were found among red clover, Ladino Clover and alfalfa. (Based on three cuts per season with Ladino Clover and two cuts for red clover and alfalfa). Less Ladino forage is required for processing to obtain a comparable quantity of protein concentrate than with alfalfa and red clover.

Treatment of fresh juice is necessary to prevent spoilage and to extend the useful life of leaf protein. Acetic acid, added to fresh juice to coagulate protein immediately, or molasses which aids in the fermentation process, help to prolong the storage life of Ladino protein concentrate. Immediate coagulation of juice protein using acetic acid results in greater yields of actual protein than where acid produced in the fermentation process causes coagulation of the protein.

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The nature of leaf protein material, which tends to be high in moisture content, poses problems with both mixing and handling of the. material. If major problems with processing, storage and nutritive value can be worked out, leaf protein would be useful as a protein supplement. Replicated weight gain tests comparing leaf protein and soybean meal as protein supplements in actual swine diets are necessary.

In summary, leaf protein concentrate of high nutritive quality may be produced in the Atlantic region. Many problems in all aspects of production, processing, storage and use must be overcome before leaf protein from forages can take its place in commercial operations.

"Within the past few years, funding for major green crop fractionation work, both in Europe and America, has ceased. Experimental results indicate major technical problems prohibit economical use of leaf protein. Green crop fractionation, at present, is not being further investigated.

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APPENDIX

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Table No. 1	Analysi	is of variance tabl	e for cut 1 dry ma	tter vield
		Ladino c	lover	
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Source	<u>df</u>	<u>55</u>	ms	<u>F cal.</u>
Total	23	17,423,264.63	1	,
Replications	3	711,901.12	237,300.37	.84
First cut date	5	12,479,858.87	2,495,971.77	8.85*
Error	15	4,231,504.64	282,100.31	E.M

*Significant at .05 level.

Table No. 2 Analysis of variance table for cut 2 dry matter yield

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Ladino clover .

Source	df	<u>55</u> .	ms	F cal.
Total	23	1,227,205.83		
Replications	3	208,409.83	69,469.94	2.48
First cut date	5	599,023.83	119,804.77	4.28*
Error	15	419,772.17	27,984.81	

*Significant at .05 level.

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	Table No. 3	Analysis of	Variance table	for cut 3 dry mat	ter yield
	•	· · ·	Ladino clor	<u>ver</u>	
		9		۰ ۲	
•	Source	<u>df</u>	<u>SS</u>	ms	F cal.
	Total	23	950,571.83 "		-
	Replications	3	41,324.16	13,774.72	•95
	First cut date	5 .	690,858.83	138,171.77	9•49*
	Error	15	218,388.84	14,559.26	
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*Significant at .05 level.

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nalysis	of ^{va} riance	summary	table dry	matter	yield
	Ladinc	o clover			
10	1				, , ,
di	, SS ,		ms		<u>F cal.</u>
.71	65,845,730.3	32			
23	5,264,956.9	9	۵		
3.	[′] 277 , 033.1	.5	92,344.3	38 ′	1.07
5 [′]	3,691,089.5	57	738,217.9	91	8.54*
15	1,296,834.2	27	86,455.6	52	
48	60,580,733.3	33		٤	
2	46,244,688.0)3 23	,122, 344.0)1 1	95-52*
10	10,078,651.9	נ קי	.,007,865.2	20	8.52*
36	4,257,433-3	33	118,252.0	04	
	df ,71 23 3. 5 15 48 2 10	Lading df ss 71 65,845,730.3 23 5,264,956.9 3. 277,033.1 5 3,691,089.5 15 1,296,834.2 48 60,580,733.3 2 46,244,688.0 10 10,078,651.9	Ladino clover df ss 71 65,845,730.32 23 5,264,956.99 3. 277,033.15 5 3,691,089.57 15 1,296,834.27 48 60,580,733.33 2 46,244,688.03 23 10 10,078,651.97 1	Ladino cloverdfssms71 $65,845,730.32$ 23 $5,264,956.99$ 3. $277,033.15$ 92,344.35 $3,691,089.57$ 15 $1,296,834.27$ 48 $60,580,733.33$ 2 $46,244,688.03$ 23,122,344.010 $10,078,651.97$ 1,007,865.2	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

*Significant at .05 level.

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Table No. 5	Analysi	ls of variance t	able for total dry	matter yïeld
,		Ladino	clover	
Source	df	SS	ms	F cal.
Total	° 23	15,794,870.96	`	
Replications	3	831,099.46	277,033.15	1.07
First cut date	5 5	11,073,268.71	2,214,653.74	8.54*
Error	15	3,890,502.79	259,366.85	5

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*Significant at .05 level.

Table No. 6	Analysis of	variance	table - cut	1 -	dry matter	yield of	
-	fresh juice	– Ladino	clover	,			
Source	df	SS		ms	F	cal.	

<u>20m.ce</u>	<u>u</u>	35	1110	r care
Total	23	181,429.33		
Replications	3	79,295.67	26,431.89	6.20*
First cut dates	5	38,250.83	7,650.17	1.80 n.s.
Error	15	63,882.83	4,258.86	
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*Significant at .05 level.

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Table No. 7	Analysis	; of variance table	cut 2 - dry	matter yield of	ر ست
	<u>fresh</u> ju	nice - Ladino clove	er	r.	
	-	,		~	
Source	df	55	ms	<u>F cal.</u>	
Total	23	28,857.96			
Replications	3	2,828.46	942.82	2.83	2
First	5	21,028.71	4,205.74	12.62*	,
Error	15	5,000.79	333.39		

*Significant at .05 level.

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Table No. 8Analysis of variance table - cut 3 - dry matter yield offresh juice - Ladino clover

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		L		
Source	<u>df</u>	<u>55</u>	ms	F cal.
Total	23	56,765.33		
Replications	3	2,091.66	697.22	•39
First cut	5	27,761.33	5,552.27	3.09*
Error	15	26,912.34	1,794.16	

*Significant at .05 level.

Table No. 9	Analysi	ls of variance su	mmary table - dry	matter yield of
-	fresh	juice – Ladino cl	over	
	U			
Source	df	<u>55</u>	ms	F cal.
Total	71	511,678.32	,	
Among Main plot units	23	117,794.32	-	ι.
Replications	3	37,145.82	12,381.94	7.64*
First cut dates	5	56,337.24	11,267.45	6.95*
Error a	15	24,311.26	1,620.75	~
Within main plot units	48	393,844.00		
Cuts	2	244,625.69	122,312.85	37-14*
Date and cuts	10	30,703.64	3,070.36	•93
Error b	36	118,554.67	3,293.19	

*Significant at .05 level.

Table No. 10	Analysis	of variance	table for total dry	matter yield
	o <u>f</u> fresh	juice	· ,	
Source	dſ	<u>55</u>	ms	F cal.
Total	23	353,382.96		
Replications	3	111,437.46	37,145.82	7.6*
First cut dates	5	169,011.71	33,802.34	6.95*
Error	15	72,933.79	4,862.25	

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*Significant at .05 level.

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Table No. 11Analysis of Variance table for cut 1 - dry matter yieldof protein concentrate - Ladino clover

Source	df	55	ms	F cal.
Total	23	40,207.63	•	
Replications	3	13,719.12	4,573.04	9-44*
First cut dates	5	19,220.37	3,844.07	7.90*
Error	15	7,268.14	484.54	

*Significant at .05 level.

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Table No. 12	Analysi	s of Variance ta	ble for cut 2 - dr	v matter vield		
at m	of protein concentrate - Ladino clover					
				~		
Source	df	SS	ms	F cal.		
Total	23	13,887.63		Ŷ		
Replications	3	1,167.45	369.15	2.49		
First cut dates	5	10,491.87	2,098.37	14.13*		

2,228.31

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148.55

*Significant at .05 level.

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Error

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Table No. 13	Analys	is of Variance tab	le for cut 3 - dry	matter yield of
, ¹	protei	n concentrate – La	dino clover	
Source	df	SS	ms	F cal.
Total	23	14,292.96		,
Replications	3,	1,303.13	434.38	.77 n.s.
First cut dates	5	4,481.21	896.24	1.58 n.s.
Error	15	8,508.62	567.24	
2			Ł	

Table No. 14Analysis of Variance summary table - dry matter yield ofprotein concentrate - Ladino clover

Source	<u>df</u>	4 <u>SS</u>	ms	F cal.
) Total	71	100,852.32		
Among main plot units	23	÷ 30,291.65		<i>\</i> :
Replications	3	8,288.26	2,762.76	8.13*
First cut dates	5	8 16,907.74	3,381.55	9. 95*
Error a	15	5,095.65	339.71	н. 1
Within main plot units	48	, 70,560.67		
Cuts	2	31,486.78	15,743.39	26.12*
Dates and cuts	10	17,379.05	1,737.91	2.88*
Error b	36	21,694.84 ©	602.63	·

*Significant at .05 level.

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Table No. 15	nalysis	of variance table	for total dry matte	er yield °
<u>(</u>	of protei	in concentrate – La	adino clover	
ý				 Q
Source	<u>df</u> .	۲ <u>. Ss</u>	ms	F/cal.
Total	23	90,874.96		
Replications	3	25,470.79	8,490.26	6.66*
First cut dates	5	46,296.21	9,259.24	7 •27*
Error	15 –	19,107.96	1,273.86	
			۴	2

*Significant at .05 level.

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Table No. 16-	Analys	is of variance table	e for cut 1 - per	centage protein
-		of protein concentr	ate — Ladino clo	ver
		١		
Source	df	<u>95</u>	ms	F cal.
Total	23	124.48	Ň	
Replications	3	63.22	21.07	7-50*
First cut dates	5	19.05	3.81	1.36 n.s.
Error	15	42.21	2.81	

*Significant at .05 level.

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14.53*

v	of pr	otein concentra	te – Ladino clover	د	
Source	df	55	. <u>ms</u>	<u>F cal.</u>	,
Total	23	27.52	``		
Replications	3	8.23	2.74	2.47 n.s.	
First cut dates	-5	2.60	•52	-47 n.s.	
Error	15	16.69	1.11		

Table No. 18	Analysis	of variance ta	ble for cut 3 - per	rcentage protein	
of protein concentrate - Ladino Clover					
		,			
Source	df	55	ms	F cal.	
Total	23	213.69			
Replications	3	4.21	1.40	.59 n.s.	
Total	23	213.69			

34.73

2.39

173.66

35.82

*Significant at .05 level.

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First cut dates 5

Error

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	of	protein concentrate	- Ladino c	lover
	<i>,</i> e,	-		"
/ Source	<u>df</u>	, <u>55</u>	ms	<u>F cal.</u>
Total	71	453 • 41	0	س می ل
Among main plot units	2 [`] 3	155.81	7	,
Replications	3	43 • 52	14.51	≠ 5•78 *
First cut dates	5	74 • 64	14.93	∘5• 95*
Error a	15	37.65	ِ 2. 51	گه
Within main plot units	48	₈ 297 • 6 0		μ, μ
Cuts	2	108.12	54.06	° 30•72*
°Dates and cuts	10	126.05	12.61	7.16*
Error b	3 6	63•43	1.76	

Table No. 39 Analysis of variance summary table - percentage protein

Significant at .05 level.

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Table No. 20 Analysis of Variance table - cut 1 - actual dry matter yield of protein (kg./ha.) - Ladino clover

Source	<u>df</u>	, <u>85</u>	ms	Fical.
Total	23	6,991.24	1	σ
Replications	້ 3	2,811.20	937.07	10.62*
First cut dates	5	2,856.93	571.39	6.48*
Error	15	·1,323.11	-88.21	· ·

*Significant at .05 level.

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Table No. 21	Analysi	s of Variance t	able - cut 2 - actu	al dry matter
· ,	vield o	f protein (kg./	ha.) – Ladino clove	<u>r</u>
	, , ,		,	, , , ;
Source 6	df	<u>88</u>	ms	F cal.
Total '	23	2,317.83	6	
Replications	3	286.16	95-39	2.96
First cut dates	5	1,548.83	103.26	3.21*
Error	15	482.84	32.19	¢
				,

*Significant at .05 level.

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Table No. 22	Analysis of variance table - cut 3 - actual dry matter

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. (معلمه	yield	of protein (kg:	/ha.) - Ladino clover	ч к
	۰ ۱	Ю	, a	AT BY	0 1
Source	' *	<u>_df</u>	. 55	ms	<u>F cal.</u>
Total		23	2,039.96	· · · ·	•
Replica	tions .	. 3	250.13	83.38	.90 n.s.
First c	ut dates	5	407.71	81.54	.89 n.s.
Error		้ 15	1,382.12	92.14	, `;

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Table No. 23	Analysis	of Variance	summary	table -	actual	dry matter
	yield of	protein (kg	./ha.) -	Ladino c	Lover	•
•		¢				, . · ·
Source	<u>df</u>	<u>88</u>	./	ms	ا م	F cal.
Total	, 71 [°]	16,675.22	, °	-	-	
Among main plot units	23	4,705-39			•	
Replications	-3	1,984.09	• •	661.37	-	8.94*
First cut dates	5	1,611.45	ι *	322.29	1 	4.36*
Error a	15	1,109.85	1	73.99	۷	f
Within main plot units	48	11,969.83	* , *	•	•	. • [`]
Cuts	2_	5,326.19	. 2	2,663.09		27.86*
Dates and cuts	10	3,202.02	,	320.20	ł	3.35*
Error b	36	3,441.62	J	95.6Ò		l l
		(a		•	ł	

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*Significant at .05 level.

Table No. 24	Analysis of	variance	table fo	or total	dry	matter yield	of.,
						*	e

actual protein - Ladino clover

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				i i	
•	Source	<u>df</u>	<u>85</u>	ms	<u>F cal.</u>
	Total	23	14,116.16		۰ ۲۰۰۰ م
	Replications	3	5,952.26	1,984.10	8.90*
	First cut dates	5	4,834.35	966.87	4.96*
	Error	15	3,329.55	221.97	

*Significant at .05 level.

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Table No. 25	Analysi	s of variance tab	ole total dry mat	ter yield comparisons		
¥ •	fromfin	from first cut of Ladino Clover, red clover and alfalfa				
, ,	(kg./ha	. dry matter)				
	,	· , 1	•	- 、		
Source	· <u>df</u>	55	. <u>ms</u>	F cal.		
Total	11	7,330,308.00				
Replications	3 .	191,500.67	63,833.56	.16 n.s.		
Species	° 2 `	4,686,048.50	2,343,024.25	5•73*		
Error	6	2,452,758.83	408 ,7 93 . 14			
	I	r		,		

*Significant at .05 level.

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Table No. 26 Analysis of variance table - juice yield comparisons from first cut material, Ladino clover, red clover and alfalfa

(kg./ha. dry matter)

			b	
Source	df	<u>.</u> <u>.</u>	* <u>ms</u>	F cal.
Total	. 11	177,628.92		t.
Replications	3	861.59	287.20	•05
Species	2	141,820 . 17	70,910.09	12.17*
Error	6	34,947.16	5,824.53	1 3
				1

*Significant at .05 level.

Table No. 27Analysis of variance table - protein concentrate yieldfrom first cut, Ladino clover, red clover and alfalfa(kg./ha. dry matter)

Source	<u>df</u>	, <u>SS</u>	- ms	F cal.
Total	11	53,522.92	a , , ,	A
Replications	3	986.25	328.75	.21
Species	Ż	43,222.17	21,611.09	<u>1</u> 3.92*.
Error	େ	9,314.50	1,552.42	,
		1		

*Significant at .05 level.

Table No. 28	Analysis of Variance table - percent Protein of Prot	ein
	Concentrate, from first cut, Ladino clover, red clov	rer .
, , ,	and alfalfa (kg./ha. dry matter)	
, ,	ann allalla (kg./lia. ury macter/	
•		

Source	<u>df</u>	<u>55</u>	* <u>ms</u>	<u>F cal.</u>
Total.	· 11	181.36	•	•
Replications	3	3.37	1.12	-28
Species	2	153.62	76.81	18.92*
Error	6	24.37	4.06	•
		r.		

*Significant at .05 Level.

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Table No. 29	<u>Analysis of</u>	variance table	actual protein	yield (kg./ha.
¢	dry matter)	from first cut	Ladino clover,	red clover and
`	alfalfa			• -
v		,		•
Source	df	<u>55</u>	ms	F cal.
Total	11	3,733.23	· ·	<i>a</i> .
Replications	. 3	226.73	75.88	•29
Species	2	1,918.04	959.02	3.62
Error	6	1,588.46	264.74	х С
	·	,	•	- -

Table No. 30 Analysis of variance table - total dry matter yield comparisons of Ladino clover, red clover and alfalfa (kg./ha.) cut at 1/10 bloom

			· .	•
Source	df	55	ms	F cal.
Total	11	10,871,112.90	- `	, · · · · · · · · · · · · · · · · · · ·
Replications	3	957,618.23	319,206.08	1.04
Species	2	8,066,467.15	4,033,233.58	13.10*
Error	6	1,847,027.52	307,837.92	,

*Significant at .05 level

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Table No. 31	Analysis of variance table - fresh juice yield (kg./ha.
•	dry matter basis) of Ladino Clover, red Clover and
, k	alfalfa cut at 1'10 bloom

Source	df	<u>88</u>	ms	F cal.
Total.	11	59,218.92		· ·
Replications	3	8,854.25	2,951.42	.44 n.s.
Species	2	10,026.17	5,013.09	•75 n.s.
Error	6	40,338.50	6,723.08	* /

Table No. 32	Analysis of	variance	e table	- protein	concentr	rate -	yield
*	(kg./ha. dr	y matter	basis)	of Ladino	clover,	red c.	Lover
0	and alfalfa	cut at]	L/10 blo	om	,	1	
			8			•	ر
Source	df	<u>55</u>		ms		F	cal.
Total	11 .	23,970.9	92			a	

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Table No. 33'	Analysis of variance table - actual	protein yield —
	v*	· · · · · · · · · · · · · · · · · · ·
۶ <u> </u>	(kg./ha. dry matter basis) of Ladir	o clover, red clover
	and alfalfa cut at 1/10 bloom	. ,

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Source	df	<u>83</u>	ms	F. cal.
Total	11	4,327.56	· · · ·	、
Replications	3,	936.39	312.13	1.10 n.s.
Species	2	1,688.37	844.19	2.97 n.s.
Error	6	1,702.80	28 3.80	. ~

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	Table No. 34	Analysis of	variance summa	ry table - tota	l yield of protein
¢		<u>concentrate</u>	using various	storage methods	(g./100g. fresh
,	ν	juice) - La	dino clover		
-	,			, ,	1
	Source	ት	°	ma	F cal.
	Domico	df	,88	ms	r car.
	Total	:47	38.53	*	
	Among main	t	*	•	
	plot units °	ʻr 15	15.31	3	
	-		· · · · · · · ·	• •	•
	Replications	3	.06	. 02	.12
	Storage treatm	,	13.07	4.57	26.86*
	Error a	· 9	1.55	.17	•
	1	1	i.		· ·
	Within main	°	- 1.87		
	plot units	<u> </u>	`23 . 22	۰	θ,
	Dates	2	° 17.03	8.52	65.50*
	Treatment & da	ate 6	3.06	.51	3.92*
		, 		1	• • ,
	Error b	24	3.13	.13	
	· -	•		•	* · ·

*Significant at .05 level

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Table No. 35	Analysis of	variance summar	y table for p	ercentage
	protein of	protein concentr	ate using var	ious storage
	<u>methods - I</u>	adino clover		¥
,				, 0
Source	df	• <u>55</u>		F cal.
Total	47	703.78	0 +	I
Among main	,		ı	i.

TUCAL	** (01.00		
Among main plot units	15	419.10	t 7) <i>ii</i> ()	1
Replications	3	5.91	1.97	•46
Storage treatment	3	374•79 ·	124.93	29.56*
Error a	9 '	38.40	4.27	
Within mạin plot units	32 ~	284.68	•	r N
Dates	2	104.35	52.18	13.66 *
Treatment & dates	6 🔒	88.71	-14.79	3.87*
Error b	24	91 ₁ 62	3.82	, ,

*Significant at .05 level.

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	· 		•		. 7. 1. 0
	Table No.36	Analysis Of Va	riance summ	ary vable - yi	eld of protein
		using various	storage met	hods (g./100g.	fresh juice) -
		Ladino clover	,	1	
	-	ر م ا	s	۴	
	Source	df	55	m <u>s</u>	F cal.
	Total	47	3.97	v,	:
	Among main plot units	15	1.41	ى	1
	Replications	3	•04	•0133	1.09
	Storage treatme	ent 3	1.26	•4200	34 • 43*
	Error a	9	.11	•0122	· ·
,	Within main plot units	32	2.56	•	
	Dates	2	1.85	•9250 °	ب 96.35*
	Treatment & dat	ces 6	.48	-0800	8.33*
•	Error b	24	•23	•0096	,

*Significant at .05 level

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Table No. 37

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Environnement Canada Environment Canada 日本 Environmement atmospherique Amospheric Environment

MONTHLY METEOROLOGICAL SUMMARY* SOMMAIRE MÉTÉOROLOGIQUE MENSUEL

JUNE / JUIN MONTH/MOIS

`1981

,			•		AT	/À	THUKC), NOV,	SCOTI	ι .	•						,		,	-
LAT 4	5 • 22	2 'N	LONG 6	3 • 1	6 W			39.9	METRES		,			NDARC BORN				tlær	ntic	•
•		MPERATUR MPERATUR			E DAYS S JOURS	REL HÚ HUMIDIT				RECIPITATI			_			WIN			·	t [*]
DATE	WATHUN MAXIMALE	MININUM	MEAN MOYENNE	HEATING DE CIIAUFFE	GROWING DE CROISSANCE	MAXIMUM MAXIMALE	KINIMUM KINIMUM	THUNDERSTORM ORAGE	RAIMFALL PLUIE (HAUTEUR)	SNOWFALL NEJGE (HAJTEUR)	TOTAL PRECIP	1	SNOW ON GROUND NEIGE AU SOL	AVERAGE SPEED		DIRECTION	MAXIVUW SPEED	VITESSE VAXIMALE		-00#5
	-16.7	5.3	11.0	BASE LAUC	0.0	97	- 38	0	<u>mm</u>	¢m.	- mu -		5	13	-1	Nn	W	281		LUALS 14. Y
2 3 4 5	20.9 22.2 19.6 25.7	1.6 8.9 12.1 11.7	11.3 15.6 15.9 18.7	0.7 2.4 2.1	6.) 10.6 10.9 13.7	100 100 100 100	32 41 77 50	00000	0.4		٥.	4	0 0 0 0		0	W SM NIVE 2011	W SSI S N.V	32 28 11 9*		12.3 10.5 1.5 13.7
6 7 8 9 10	22.1 17.4 16.5 19.6 11.1	10.4 10.4 8.0 8.1 8.7	16.3 13.9 12.3 13.9 9.9	1.7 4.1 5.7 4.1 8.1	11.3 8.9 7.3 8.9 4.9	100 100 95 100 100	57 83 50 57 83	000000000000000000000000000000000000000	1.6 1.8 29.4 0.4		1. 1. 29. 0.	8	0 0 0 0		9	s W Svrl W	. w	194 224 43 37 41	•	6.6 0.2 14.3 0.6 0.0
11 12 13 14 15	15.0 16.2 16.9 20.7 15.3	7.3 6.8 7.8 7.5 8.7	11.2 11.5 12.4 14.1 13.5	6.8 6.5 5.6 3.9 ~ 4.5	6.2 6.5 7.4 9.1 8.5	100 100 100 100 100	73 54 63 49 52	000000	TR 7.6		TR 7.		00000	3	.0 .1 .2	NOW E SE S W	SE SE	11 11 11 11 22*		2.9 3.6 2.8 4.5 3.2
16 17 18 19 20	16.2 22.3 22.2 26.0 24.5	12.0 12.8 8.1 6;2 12.5	14.1 17.6 15.2 16.1 18.5	3.9 0.4 2.8 1.9	9.1 12.6 10.2 11.1 13.5	100 100 100 100 100	83 87 25 33 47	00000	3.2 0.2 4.2		3. 0. 4.	2	0000000	6 9 9	3 5 8 0	ร พ ม ม	W N N S	28 19 28 28* 17*	- 1	0.0 1.0 14.8 14.8 14.8 8.8
21 22 23 24 25	19.1 22.9 23.5 21.2 19.3	11.0 10.8 9.8 7.0 9.2	15.1 16.9 16.7 14.1 14.3	2.9 1.1 1.3 3.9 3.7	10.1 11.9 11.7 9.1 9.3	100 100 100 100 100	82 45 56 35 36	00000	29.8 6.0 17.2 13.8		29. 6. 17. 13.	2	0000		5	S SVRL SVRL W S	SE W	13* 20 33* 28 19	1	0.0 9.2 13.3 12.8 1.4
26 27 28 29 30	21.5 20.5 23.2 22.6 26.6	11.7 8.2 0.5 5.5 12.0	10.6 14.4 14.9 14.1 19.3	1.4 5.5 3.1 3.9	11.0 9.4 9.9 9.1 14.3	100 100 100 100 100	82 45 34 41 52	0 0 0 0	5.0		5.	0	0 0 0 0	8	اد	Sje N N ENE SVRL	551 7 × 12 53	17 19 24		4.0,0
31				TOTAL	TOTAL	<u> </u>		TOTAL	TOTAL	TOTAL	TQTA					1 VAIL 15:	' '	1.2		1014
MEAN I MOYENHE	20.4	8,9	14.7	103.1	289.4	100	55	0	120.6	0	120					¥6 000000001		43		19.2
NORNAL	19.4	7.2	13.3	138.5	264.6			2	62.7	Nil	02.			13.	<u> </u>	W				21.2
		DE	GREE DAY		- 50MMA	IRE DE DEC	RESJOUR	s 	r 7	·	101	JRS AV	IC FALCH	NTATIO	43	700	AS AVE	6 CHUII 6 CHUII	E Dê Ni	ii61
	DW 18"C DUS DE 11"C	Turs Yeak Anntii En COURI	Phtvic VEAJ Abot PAIctor	a ad t bo	я рац 1маце	ABOVE AU DESSUS	,	THIS YEAR ANNÉE EN COURS	PIEVICUS VIAN ANUE PRECEDENTE	BORMAL BORMALS	0 7 83 4075	1 0 GA WORE	20 QA	10 0 01 10 10	50 0 DA MORI	0 7 01 HQAE	1 0 0A 100AE	2 0 0A NORE	10 0 DR 10 0	50 0 68 1071
	OR MONTH	103.1	154	.6 13	8,5	TOTAL FOA TOTAL DU		289,4	242.0	264.6	OU PLUS	51 01	OU PLUS	0U 71.US	00 P[U3	OU PLUS	00 PLUS	OU PLUS	0U 91.US	PLUS
SINC ACCU	IULATED E JULY 1 WULEE IV JUILLET	4592.9	4634	.9 465	7.7	ACCUMUL SINCE API ACCUMU DEPUIS LE 1	NIL 1	513.1	377:3	384.4	14	11	9	4	0	0	0	0	0	0

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ACCUMULATED SINCE JULY 1 ACCUMULEE DEPUIS LE 1º JUILLET

but not necessarily the same direction.

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Table No. 38

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14	Environment Canada	Environgement Canada
,	Atmospheric	Environnement atmosphérique

MONTHLY METEOROLOGICAL SUMMARY SOMMAIRE MÉTÉOROLOGIQUE MENSUEL

MONTH/MOIS JULY/JULLET

1981

AT/À TRURO, NOVA SCOTIA

LAT. 4	5 · 22	'N	LONG 6	3·16	w	E	LEVATIO	N 39.9	METRES MÈTRES		STANDARD TIME USED NEUME NORMALE UTILISÉE Atla								Lie	
		WPERATUR MPERATUR		DEGRE	E DAYS S JOURS	REL HU				RECIPITAT			_	V V			WIND		1	
DATE	MAXIMUM	MINIMALE MINIMALE	MEAN	HEATING DE CHAUFFE	CROWING DE CROISSANCE	, Maximum Maximale	HININUM HININALE	THUNDERSTORM ORAGE	RAINFALL PLUIE (HAUTEUR)	SNOWFALL NEIGE (MAUTEUR)	TOTAL PRECIP.	PRECIP TOTALE	SNDW ON GROUND NEIGE AU SOL	AVERAGE SPEED	VITESSE MOYENNE	DIRECTION	MAXIMU4 SPEED	VITESSE MAXIMALE ET DIRECTION	A BRIGHT SUNSHIK A BRIGHT SUNSHIK INSOLATION EFFECTIVE	
		<u>۲</u>	<u>``</u>	11.28 18.0°C	8458 54		3			6m		<u>~</u>	um D	10	_	SYHL	1-	1.00/11	NEURES	
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[08	GREEDAY	SUMMARY	- SOMM	AIRE DE DEG	RES-JOU	RS	·	,	01	URS AV	IC PRÉCI IOTALES mm	DITATIC	7#5	UOL	ns Ave	C CHUTE	DE NEIGE	
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Table No. 39

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MONTHLY METEOROLOGICAL SUMMARY SOMMAIRE MÉTÉOROLOGIQUE MENSUEL

MONTH/MOIS AUGUST ./ XOUT

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MONTHLY METEOROLOGICAL SUMMARY SOMMAIRE MÉTÉOROLOGIQUE MENSUEL

MONTH/MOIS SEPTEMBER/SEPTEMBRE TRURO NOVA SCOTIA

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