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A COMPARISON OF THE NUTRITIONAL VALUE OF
NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

W. J. Pigden

Department of Plant Science

University of Alberta

Thesis
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U N I V E R S I T Y   O F   A L B E R T A

FACULTY OF AGRICULTURE
DEPARTMENT OF PLANT SCIENCE

The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, "A Comparison of the Nutritional Value of Native and Introduced Grasses for Winter Pasture", submitted by Wallace James Pigden, B.S.A., in partial fulfilment of the requirements for the degree of Master of Science.

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Date

May 8, 1950

UNITED STATES DEPARTMENT OF AGRICULTURE

OFFICE OF THE ASSISTANT SECRETARY FOR TECHNICAL AND ECONOMIC RESEARCH

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REPORT

BY

THE

THE UNIVERSITY OF ALBERTA

A COMPARISON OF THE NUTRITIONAL VALUE OF
NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

by

Wallace James Pigden

EDMONTON, ALBERTA

MAY, 1950

THE UNIVERSITY OF CHICAGO

 

BY

WILLIAM J. BROWN

CHICAGO, ILLINOIS

MAY, 1950

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A COMPARISON OF THE NUTRITIONAL VALUE OF NATIVE AND INTRODUCED GRASSES FOR WINTER PASTURE

W. J. Pigden

INTRODUCTION

The livestock industry is an important branch of Agriculture in the three Prairie Provinces, Manitoba, Saskatchewan, and Alberta. Figures supplied by the 1948-49 Canada Year Book, indicate that the livestock population numbered some 3,944,000 cattle, 1,080,000 sheep, and 1,114,000 horses, in 1947. According to Clark and Tisdale (7), the major portion of the forage requirements for these animals is supplied by native pasture lands which have been estimated to occupy at least forty million acres.

A considerable proportion of this acreage is utilized for its cured forage during the fall and winter months. This is possible for two main reasons:

- (1) The warm chinook winds which usually partially clear the snow from the pastures and moderate the temperatures in many areas.

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REPORT

The livestock industry is an important branch of agriculture in the three Prairie Provinces, Manitoba, Saskatchewan, and Alberta. It is one of the leading sources of wealth in these provinces. The livestock population numbered 2,500,000 cattle, 1,000,000 sheep, and 1,100,000 horses in 1911. According to the 1911 census, the major portion of the livestock population for these animals is owned by native persons. It has been estimated that nearly 10 million acres.

A considerable proportion of this livestock is killed for the cured meats during the fall and winter months. It is possible for two reasons:

- (1) The very cold winds which usually prevail during the snow from the pastures and herds the temperature in

many areas.

- (2) Several grasses common to the prairies of western Canada exhibit to a marked degree a property arbitrarily designated as "curing". Curing is defined as the process which conditions the plant so that after growth ceases it:
- (a) retains a relatively high nutritive value during late summer and throughout the subsequent fall and early winter,
  - (b) preserves its physical form, stems and leaves do not become excessively hardened, neither do they lacerate or decompose to any extent for some 8 to 10 months after the curing process occurs. Curing normally takes place during late July but may be as early as mid June, or as late as the latter part of August, depending on the season.

Ranchers report that curing results in a forage which maintains sheep and cattle in a thrifty condition during the winter with little or no supplemental feeding, despite the fact that protein content of the cured forage is usually less than five per cent (7). Hence, from the viewpoint of the rancher, curing is an invaluable asset - it decreases production costs by shortening materially the winter feeding period.

As stated previously, the bulk of the forage for livestock production on the Prairie is derived from native grass

(8) Several species common to the forests of Western Canada exhibit to a marked degree a property which is designated as "caring". Caring is defined as the process which com-

ditions the plant so that after growth ceases it:

(a) retains a relatively high relative value during late

summer and throughout the subsequent fall and early

winter,

(b) preserves its physical form, leaves and stems do not

become excessively hardened, neither do they lose their

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the caring process occurs. Caring normally takes place

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Researchers report that during winter in a forest where

maintains a very high and stable in a healthy condition during the

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that protein content of the animal tissue is usually less than

five per cent (7.5 per cent). Hence, from the viewpoint of the animal, winter

is an invaluable asset - it decreases metabolic costs of winter-

feeding, especially the winter feeding period.

As stated previously, the aim of the study for five-

stock production on the prairie is derived from native grass

pastures. An increasing acreage of unproductive land - depleted pastures, submarginal cultivated lands and abandoned farms - is however, being regrassed with comparatively high yielding introduced grasses (6).

Several of these grasses, now being extensively used for this purpose, appear to lack, wholly or in part, the curing property. This deficiency tends to limit their usefulness in a production program where feed reserves are normally accumulated in the form of winter pasture. Therefore it would be highly desirable to obtain a grass which would incorporate the curing property - as exemplified by the most valuable native grasses - with high yield, winter hardiness, drought resistance and other desirable characteristics displayed by certain cultivated species.

The first logical step in the development of such a superior grass appears to be a study of the factor or factors involved in the curing process. Once this knowledge has been obtained, the plant breeder can decide whether the information can be applied to the development of improved types of forage for winter pasture.

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obtained, the plant breeder can decide whether the information
can be applied to the development of improved types of grasses
for winter pastures.

The following native grass species are credited with the ability to cure to a greater or lesser degree:

<u>Common Name</u>	<u>Scientific Name</u>
1. Common speargrass	<u>Stipa comata</u> Trin. and Rup.
2. Blue grama grass	<u>Bouteloua gracilis</u> (H.B.K.) Lag.
3. Western wheatgrass	<u>Agropyron Smithii</u> Rydb.
4. Rough fescue	<u>Festuca scabrella</u> Torr.
5. Parry's oatgrass	<u>Danthonia Parryi</u> Scribn.
6. Short awned porcupine grass	<u>Stipa spartea</u> var. <u>Curtiseta</u> Hitchc.
7. Niggerwool	<u>Carex filifolia</u> Nutt.
8. June grass	<u>Koeleria cristata</u> Pers.
9. Northern wheatgrass	<u>Agropyron dasystachym</u> (Hook) Scribn.

In addition several grass species of minor importance as well as certain forbes, particularly salt sage, Atriplex nuttallii S. Wats. and winterfat, Eurotia lanata (Pursh) Moq., may have this property.

These species are dominant in the prairie area within the provinces of Manitoba, Saskatchewan and Alberta. The foothill area on the west and as far north and east as the southern boundary of the Park Belt is included within the region of distribution.

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Preliminary investigations were started in 1948 at the Dominion Experimental Station, Swift Current, Saskatchewan. Two native grasses (known to cure well) and two cultivated grasses (non-curing, or to a limited degree) were selected for comparative studies. Specimens of each grass were collected, mounted and photographed.

A comprehensive literature survey was carried out at the University of Alberta, 1948 - 49, to determine what investigational methods would be followed. As a result it was decided to study the problem from both a biochemical and histological standpoint with special reference to lignin.

The biochemical analysis was designed to lend support to, or disprove, two main hypotheses:

1. Lignin content:

Since lignin is almost indigestible, a high lignin content would probably markedly lower the nutritive value of cured forage.

2. Solubility of reserve carbohydrates:

A readily water soluble reserve substance such as sucrose - commonly found in the Gramineae - would be leached out of the dried tissues much more readily than a less soluble carbohydrate reserve such as starch.

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The histological study was intended to supply a general overall picture of the anatomy of leaf and stem, with special reference to the position and extent of the lignified tissues. It was hoped to determine whether any relation existed between these features and the curing habit.

Most of the histological study was carried out at the Dominion Experimental Station, Swift Current, during the summer of 1949. The biochemical analyses were also started there in the Soils Laboratory and were completed in the laboratories of the Department of Plant Science, University of Alberta, 1949-50.

The histological study was made on a series of sections

of the tissue of the embryo of *Salmo gairdneri*, taken at various stages of development, and the results are given in the accompanying figures. It was found that the histological changes which take place in the embryo of *Salmo gairdneri* during its development are similar to those which take place in the embryo of *Salmo gairdneri* during its development.

For the histological study, the embryos were fixed in Bouin's fluid, and the sections were stained with hematoxylin and eosin. The histological changes which take place in the embryo of *Salmo gairdneri* during its development are similar to those which take place in the embryo of *Salmo gairdneri* during its development. The histological changes which take place in the embryo of *Salmo gairdneri* during its development are similar to those which take place in the embryo of *Salmo gairdneri* during its development. The histological changes which take place in the embryo of *Salmo gairdneri* during its development are similar to those which take place in the embryo of *Salmo gairdneri* during its development.

LITERATURE REVIEW

Insofar as the writer is aware, there are no published data on studies dealing with the curing ability of the native grasses of western Canada. This literature survey is therefore of necessity based on information derived from more indirect sources, mainly in the fields of animal nutrition and plant physiology.

Clark and Tisdale (7) have reported the chemical analysis of approximately 1,000 samples of native vegetation from the area concerned. Data on the chemical composition of the most important cultivated species within this area are also available (7, 32). Unfortunately this information, as supplied by standard chemical feedstuffs analysis, does not show differences of sufficient magnitude to account for the superior nutritive value of cured native grass forage as compared with cultivated grasses. This is not surprising, however, as many of our leading animal nutritionists have repeatedly demonstrated serious shortcomings in the present system of feedingstuffs analysis, particularly the division of the carbohydrates into crude fibre and nitrogen-free extract (Weende Method), (43).⁶

LITERATURE REVIEW

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Investigations have shown that frequently much of the lignin, a relatively indigestible constituent of feeds, is included with the nitrogen-free extract, the supposedly readily digestible fraction (46, 47). Conversely, crude fibre, which is normally considered to be the woody, poorly digested portion, may have a coefficient of digestibility fully equal to that of the nitrogen-free extract (11, 47).

Substitution of the crude fibre and nitrogen-free extract proximate analysis by determinations for lignin, cellulose and other carbohydrates, has been advocated by Crampton and Maynard (11). For research purposes, particularly where digestibility trials are involved, many investigators now carry out parallel determinations using both methods. Most of the evidence from such trials indicates that the results using the proposed modification agree much more closely with actual digestibility studies than do those using the Weende Method (9, 10, 11, 20, 27, 35, 39) hence it seems likely that it would provide a more accurate index of the actual nutritional value of a given feed.

Since the development and use of these improved analytical techniques, lignin has been recognized as an increasingly important factor in determining the utilization of forages. For this reason it is dealt with here, in detail.

Investigations have shown that the amount of nitrogen, a relatively high percentage of food, in the animal's diet is nitrogen-free extract, the apparently readily digestible fraction (16, 17). Conversely, crude fibre, which is normally considered to be the woody, poorly digestible portion, may have a coefficient of digestibility only equal to that of the nitrogen-free extract (11, 17).

Substitution of the crude fibre and nitrogen-free extract proximate analysis by determinations for 11 ml, cell-wall and other carbohydrates, has been advocated by Crumpton and Latham (11). For practical purposes, particularly where digestibility trials are involved, such investigators now carry out parallel determinations using both methods. Most of the evidence from such trials indicates that the results using the proposed modification agree much more closely with actual digestibility studies than do those using the former method (9, 10, 11, 20, 21, 22, 23, 24, 25). Hence it seems likely that it would provide a more accurate index of the actual nutritional value of a given food.

Since the development and use of these improved analytical techniques, lignin has been recognized as an important factor in determining the utilization of forages, for this reason it is dealt with here in detail.

The chemical structure of lignin is still obscure. Although it is included in the carbohydrate fraction in the routine feedstuffs analysis, it is not a true carbohydrate since it is known to be an aromatic compound containing methoxyl groups (2). Whatever its structure may be, its main significance as a constituent of feeds lies in its relatively indigestible nature.

The digestion coefficients for lignin vary considerably depending on species of animal concerned, species of plant and chemical method employed for its isolation (10, 11, 13, 21, 22, 24, 37, 42, 57, 58). Most of these coefficients fall within the range from zero to 20 per cent, but Bondi and Meyer (1) have claimed the unusually high values of 35.1 to 64.0 per cent for the digestibility of lignin in various Egyptian forages. Most of the more recent evidence, however, supports the contention that lignin is relatively indigestible, so much so that Forbes and Garrigus (22) have found it to be a useful marker for computing the dry matter intake of grazing animals. In any event, according to Crampton (8) the small amounts of lignin which are digested are of little dietary value to the animal since most of it is excreted in the urine.

Numerous research workers have indicated that the digestibility of feedstuffs is directly related to their lignin content. Crampton (9) and Crampton and Forshaw (10) carried out digestibility

The chemical composition of lignin is still unknown.

Although it is known that lignin is a complex of various chemical groups, it is not yet possible to determine its exact composition. It is known that lignin is a complex of various chemical groups, it is not yet possible to determine its exact composition. It is known that lignin is a complex of various chemical groups, it is not yet possible to determine its exact composition.

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trials with rabbits using pasture herbage clipped at various stages of maturity. They found that the lowest growth rates and poorest digestibilities coincided with the herbage samples highest in lignin. Forbes and Garrigus (22) using steers, found dry matter digestibility and total digestible nutrient content of various forages varied inversely with lignin content of the forage. Drapala et al (15) used sheep to test the digestibility of several cuttings of red clover at various growth stages. These workers concluded on the basis of their chemical analyses, that the substantial decreases in digestibility coefficients which took place between cuttings two and four could only be accounted for by small increases in lignin content. The digestibility of crested wheatgrass cut at various stages of maturity was tested in Montana with Hereford steer calves (4). The results indicated that highest lignin values were associated with lowest gains in weight. Lancaster (33) found a very high negative correlation of -0.98 between lignin content of 14 feeds (hays and succulents) and the digestibility of their organic matter by sheep. Phillips and Loughlin (53) found a high negative regression between per cent lignin and digestibility for alfalfa and timothy hays.

Because pasture grasses have a relatively high lignin content depending on stage of growth, species, climatic conditions and other factors, lignin is of particular interest to the pasture

trials with results using pasture samples of various stages of maturity. They found that the lowest growth rates and poorest digestibilities coincided with the lowest lignin content. Highest is lignin. Forster and Germon (22) using sheep, found that matter digestibility and total digestible nutrient content of various forages varied inversely with lignin content of the forage. Trials at AI (17) used sheep to test the digestibility of several cultivars of red clover at various growth stages. These workers concluded on the basis of their chemical analyses, that the substantial decrease in digestibility coefficients with time from place between cutting two and four could only be accounted for by small increases in lignin content. The digestibility of crested wheatgrass cut at various stages of maturity was tested in Montana with Hereford steer calves (4). The results indicated that highest lignin values were associated with lowest gains in weight. Lancaster (23) found a very high negative correlation of -0.95 between lignin content of 14 weeds (grasses and subshrubs) and the digestibility of their organic matter by sheep. Williams and Houghlin (24) found a high negative regression between per cent lignin and digestibility for alfalfa and timothy hays. Because pasture residues have a relatively high lignin content depending on stage of growth, species, climatic conditions and other factors, lignin is of particular interest to the researcher

biochemist. A large number of investigations have established the fact that in general lignin content of forage plants increases steadily with advancing maturity. Patton and Gieseke (51) found that the lignin content of five Montana grasses from two localities increased steadily as the season advanced from about five per cent in May, to 18 per cent in September. The following season, Patton (50) reported a similar relationship for grasses from the same plantings and four additional species as well. The only exception was glue grama grass (*Bouteloua gracilis*) which had a surprisingly high lignin content at an early vegetative stage. Drapale et al (15) reported the lignin content of red clover for two seasons ranged from 7.3 per cent to 15.2 per cent for the first and fifth cuttings respectively. Other workers in this field, Crampton (9), Crampton and Forshaw (10), and Norman (48), have all published similar findings for numerous grasses, legumes and mixed herbages.

That wide variations exist in lignin content between species and between varieties is also known. According to Patton and Gieseke (51), mountain brome grass (*Bromus marginatus*) Nees. and Fairway crested wheatgrass (*Agropyron cristatum*) (L.) Gaertn., have a much lower lignin content than smooth brome grass (*Bromus inermis*) Leyss., and Standard crested wheatgrass respectively.

Lignin content of forages is also influenced by environmental factors which adversely affect growth. Patton and Gieseke (51) showed that grasses from drier regions of Montana reached

their maximum lignin content earlier in the season. Maynard (41) reviewed the findings of several workers in this regard and reported that unusually dry or cold weather apparently causes abnormal increases in lignin content at earlier growth stages.

Important as the quantitative influence of lignin on digestibility appears to be, there is good reason to believe that still more important principles may be involved. Crampton and Forshaw (10) noted a marked decrease in the digestibility of pasture herbage over the short period of ten days with only small increases in lignin content. These workers suggest that at a certain stage of growth, mode of deposition in plant tissues may be more important than the actual increase in lignin content. Evidence to support this assumption is not entirely lacking. Drapala et al (15) applied histological methods to a study of the process of lignification in red clover and made the following observations:

1. Lignification intreases steadily with advancing maturity starting at the base of the plant, and proceeding upward.
2. The regions around the vascular bundles are mainly involved, the xylem itself becoming more heavily lignified and sclerenchyma tissue forming in the immediate area.
3. Xylem parenchyma underwent lignification at maturity thus making the food reserves in these cells partially or wholly unavailable to the animal.

their maximum in the constant section in the second half of the experiment. (11) reviewed the findings of several workers in this field and reported that normally one or both vascular bundles increase in lignin content as the plant grows older. It is important as the quantitative influence of lignin on digestibility appears to be, there is good reason to believe that still some important principles may be involved. For example (12) noted a marked decrease in the digestibility of pasture herbage over the short period of ten days with only a slight increase in lignin content. These workers suggest that at a certain stage of growth, most of the lignin in the plant is located in the vascular bundles and the amount of lignin in the vascular bundles is not entirely lacking. Evidence to support this assumption is not entirely lacking. (13) at (14) applied statistical methods to a study of the effects of lignification in the leaves and stems of the following observations:

1. Lignification increases steadily with increasing maturity of the plant, and preceding ripening.
2. The regions around the vascular bundles are highly lignified, the xylem itself becoming more heavily lignified and sclerenchyma tissue forming in the inter-bundle areas.
3. Xylem parenchyma undergoes lignification at maturity, but the food reserves in these cells partially or wholly are available to the animal.

Additional information was gained on the behaviour of lignified tissues in the alimentary tract of the sheep by studying slides prepared from feces residues (15). These slides indicated that the general increase in the number and size of the pieces of undigested plant tissue was directly related to higher lignin content and advancing maturity of the red clover forage.

These important observations indicate that most of the lignin, in some forages at least, up to a certain stage of maturity is deposited mainly in the vascular bundles. At this point lignin gradually begins to infiltrate and encrust the walls of adjacent tissues and a marked drop in the digestibility of the forage is likely to occur.

The detailed manner in which increasing lignification affects the digestibility of forages is believed to be as follows: As the cell wall develops and thickens, lignin and hemicellulose are deposited along with cellulose to give interpenetrating systems (48), the lignin acting as a physical barrier to prevent the decomposition of cellulose (41, 48). Unless the cellulose cell wall is broken down, it in turn seems likely to hinder the action of enzymes on the more digestible nutrients such as protein, fat, starch, etc. (41, 48).

Irrespective of the degree to which lignin reduces the availability of the readily digestible nutrients, its adverse

In addition, the fact that the rate of digestion of
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follows: As the cell wall develops and thickens, lignin and
cellulose are deposited along with cellulose to give inter-
penetrating systems (16). The lignin acting as a physical
barrier to prevent the decomposition of cellulose (17, 18).
Unless the cellulose cell wall is broken down, it is then
likely to hinder the action of enzymes on the more digestible
nutrients such as protein, fat, starch, etc. (19, 20).
In respect of the degree to which lignin content
the availability of the readily digestible nutrients, its

effects on the utilization of cellulose as such, is an extremely important factor. The value of cellulose as a feedstuff may be realized when we consider that in mature forages it comprises nearly one-half of the total carbohydrates which make up 60 - 85 per cent of the dry weight of the plant (41). The breakdown of plant cellulose to simpler products is accomplished by the microorganisms which inhabit the alimentary tract of herbivorous animals. Although cellulose cannot be expected to provide the animal with as much absorbable energy per pound as is provided by the enzymic digestion of starch, because of the loss of gases in the fermentation process (40), it does provide a large part of the energy derived from roughages.

Several European workers have shown that the digestibility of coarse roughages is greatly enhanced by partial removal of the lignin, which can be accomplished by a pre-treatment with dilute alkali. Ferguson (21) concluded that alkali treatment of good wheat straw approximately doubled its feeding value to the ruminant. Homb (29) claims that treatment with sodium hydroxide, increased the coefficient of digestibility of the organic matter of straw from 42.4 to 63.7. Watson (59) reports that in England during the last war considerable amounts of wheat and barley straw were treated in

effects in the utilization of carbon dioxide as shown in the following table. The value of utilization as a percentage may be realized when we consider that in nature it comprises nearly one-half of the total energy produced which takes up to 80 per cent of the dry weight of the plant (11). The breakdown of plant utilization to display growth is accomplished by the microorganisms which furnish the assimilatory tract of herbivorous animals. Although cellulose cannot be expected to provide the animal with an immediate energy per pound as is provided by the simple digestion of sugar, because of the loss of gases in the fermentation process (12), it does provide a large part of the energy derived from vegetation.

Several European workers have shown that the digestibility of certain roughages is greatly enhanced by partial removal of the lignin, which can be accomplished by a treatment with dilute alkali. Watson (13) concludes that alkali treatment of rough feed straw appreciably increases feeding value to the ruminant. Ford (14) claims that treatment with sodium hydroxide, increased the utilization of digestibility of the organic matter of straw from 12.4 to 22.7. Watson (15) reports that in England during the last war considerable amounts of waste and scrap straw were treated in

this manner to improve their digestibility.

Cellulose in a relatively pure form such as that isolated from plants is claimed to be highly digestible by ruminants (35, 36, 38). On the other hand, cellulose associated with lignin and other substances in forages is believed to be digested to a much smaller extent (21, 35, 36, 38). According to Norman and Fuller (49) "since lignification is a process of maturity, there are concurrent changes in composition which must be taken into account, such as a reduction in protein content and in water-soluble constituents. When due allowance is made for these, however, it is found that the cellulose of a material containing five per cent lignin is far more easily attacked than the cellulose of a material containing fifteen per cent."

Other factors which have a definite bearing on the availability of plant nutrients in cured forage are soluble carbohydrates such as sugars, protein, and ether extract. Each of these is briefly discussed in turn.

According to Guilbert and Mead (25) range cattle in the California foothills fatten readily on bur clover immediately after it cures on the stem. They also observed that if heavy rains occurred at this stage the high nutritive content of this plant was frequently reduced to a sub-maintenance level within

this manner to improve their digestibility.

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Other factors which have a definite bearing on the

availability of plant nutrients in crop forages are soil

carbohydrates such as sugars, protein, and other extracts. Some

of these are briefly discussed in Table I.

According to Hillebrand and Jones (43) "the energy in

the California forage is largely derived from the energy available

after it comes on the stem. The data showed that it is

mainly because at this stage the high nutritive content of this

plant was frequently reduced to a sub-maintenance level when

a few days. Subsequent experiments indicated that significant decreases in digestibility of the cured forage occurred when the bur clover was exposed to sunlight and rain. These workers also found that as much as 35 per cent of the nitrogen-free extract and up to 16 per cent of the crude protein could be removed from bur clover by artificial leaching in the laboratory (26). Norman (48) has reported that as much as 34 per cent of a water soluble fructosan occurred in rye grass Lolium italicum var. Western Walths, in England at an early growth stage. Morosov (45), working in Russia, found over 20 per cent of inulin-like substances presumably fructosan in the stems of smooth brome grass at a mature stage. Le Clerc and Breazeale (34) experimented with wheat, oats, and various other plants and reported that the largest losses of soluble nutrients by leaching occurred when the plants reached maturity. These data suggest that considerable losses in nutrients, especially carbohydrates, may occur if they are present in a water soluble form at the mature stage.

Translocation of soluble reserves from the aerial parts of the plant to the roots late in the season may also be a factor. Rapp (54) found that Johnson grass stored most of its carbohydrate reserves in the stem during the growing season as sucrose and transferred it to the roots at maturity.

a low degree. Independent experiments indicated that significant decreases in digestibility of the clover were observed when the clover was exposed to sunlight and rain. These workers also found that as much as 3% per cent of the nitrogen in the extract and up to 15 per cent of the crude protein could be removed from the clover by artificial leaching in the laboratory (25). Norman (26) has reported that as much as 3% per cent of a water soluble nitrogenous substance occurred in the grass *Polypogon monspeliensis* in England at an early growth stage. Norman (26), working in Canada, found over 20% per cent of water-soluble substances in the stems of clover plants at a young stage. He also and previously (27) experimented with wheat, oats, and various other plants and reported that the largest losses of soluble nutrients by leaching occurred when the plants reached maturity. These data suggest that considerable losses in nutrients, especially carbohydrates, may occur if they are present in a water soluble form at the mature stage.

Translocation of soluble reserves from the aerial parts of the plant to the roots late in the season may also be a factor. Kopp (28) found that Johnson grass stored most of its carbohydrate reserves in the stem during the growing season as sucrose and transferred it to the roots at maturity.

Since the protein content of forages almost invariably declines with advancing maturity a part of the usual decrease in digestibility which occurs as the season progresses can undoubtedly be attributed to this factor. This important aspect of the field of nutrition will not be discussed here; rather it is desired to call attention to the very low protein content of cured native grasses (7), which obviously contributes relatively little towards the maintenance requirements of the range animal. The popular concept has been that rations consisting of roughages with a very wide nutritive ratio require a protein supplement for efficient digestion of the roughage (40). There is some evidence to suggest that the protein requirement for efficient roughage utilization is extremely low unless starch or starchy grains are also included in the ration (3).

According to Louw et al (36) old dry winter grazing in South Africa with about three per cent protein, is apparently not deficient in nutrients required by ruminal organisms responsible for the breakdown of cellulose to a limit presumably set by the degree of lignification.

The digestibility and energy value of the ether extract (crude fat) fraction of forages is usually fairly low

Since the general content of the paper is not
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roughage (40). There is some evidence to suggest that the
protein requirement for efficient roughage utilization is ex-
tremely low, since starch or starchy grains are also included
in the ration (2).

According to Low et al (36) old dry winter grazing
in South Africa with about 500 gms per cent protein, it apparently
not sufficient in nutrients required by normal organisms res-
ponsible for the breakdown of cellulose to a limit presumably
set by the degree of lignification.

The digestibility and energy value of the ration
extract (crude fat) fraction of forage is usually fairly low

in comparison to the ether extract of seeds (40). This is because the extracting solvent removes from the plant material, in addition to the true fats, i.e. esters of fatty acids, chlorophyll, xanthophyll, carotene, waxes and other ether soluble substances which may amount to from 25 to 40 per cent of the total ether extract of leaf material (40). As a group, these non-lipid materials are very low in digestibility and tend to depress the digestibility of the ether extract as a whole (27, 40).

Several factors that are believed to influence the nutritive value of cured forages have been reviewed. Lignin is believed to be the most important.

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Several factors that may affect the digestibility of
nutritive value of these materials have been reviewed. It is
believed to be the most important.

MATERIALS AND METHODS

Grasses Studied

Since this investigation was regarded as being more or less of a preliminary nature, the grasses were selected to represent a fairly wide range of grass types found under various growing conditions. In this manner it was hoped to gain general information which would be of value for planning future investigations. The following grasses were chosen for comparative studies.

Native

- (1) Common speargrass Stipa comata Trin. and Rup.
- (2) Rough fescue Festuca scabrella Torr.

Cultivated

- (1) Crested wheatgrass (Standard variety) Agropyron cristatum (L.) Beauv.
- (2) Common or smooth brome grass Bromus inermis Leyss.

APPENDIX A

APPENDIX B

Since this investigation was conducted on a basis of or less of a preliminary nature, the results were selected to represent a fairly wide range of cases typical of the conditions prevailing. In this regard it was hoped to call attention to the information which would be of value for planning future investigations. The following results were chosen for comparative studies.

Results

- (1) Common symptoms: Stiff neck, Stiff arms, and Stiff legs.
- (2) Stiff neck, Stiff arms, and Stiff legs.

Conclusions

- (1) Stiff neck, Stiff arms, and Stiff legs (all three symptoms) are common.
- (2) Common or moderate symptoms of Stiff neck, Stiff arms, and Stiff legs.

These four grasses are illustrated (Figs. 1 to 4) and described briefly on the next four pages.

In addition, two grasses of minor importance found on the common speargrass site, namely sandgrass Calamovilfa longifolia (Hook) Scribn. and Indian ricegrass Oryzopsis hymenoides (R. and S.) Ricker were included in the histological study but not in the chemical analyses.

Hereafter the grasses will be referred to in the text as speargrass, fescue, crested wheatgrass, brome grass, sandgrass, and ricegrass.

These four species are illustrated (figs. 1 to 4) and described

briefly on the next four pages.

In addition, the presence of other important forms

on the common occurrence site, namely Leptocarpus

lanceolatus (Lach.) and Leptocarpus lanceolatus

lanceolatus (Lach.) and Leptocarpus lanceolatus

are not in the chemical analysis.

Therefore the species will be referred to in the text

as Leptocarpus, lanceolatus, lanceolatus, lanceolatus, lanceolatus

and lanceolatus.

Fig. 1

Common speargrass
Stipa comata (native)

Speargrass is an extremely xerophytic grass, the second dominant in the shortgrass prairie and the dominant species in the mixed prairie association (5). Because of its relative abundance, palatability, and marked ability to cure, it is very highly regarded as a winter forage in the Prairie region.

This specimen shows greater than average production of seed stalks, both in number and vigor. It is estimated that during a normal year at least 75 per cent of the cured forage (dry weight), would consist of leaves, mainly basal leaves. Frequently the ratio of leaf to stem is considerably higher as was the case in 1949, Table 1.

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Fig. 2

Rough fescue
Festuca scabrella
(native)

Rough fescue is a less xerophytic grass than speargrass favoring a cooler climate with better moisture conditions. For this reason it is found only in favored locations in the mixed prairie area around Swift Current. It is the dominant species in the submontaine prairie association (5), producing an abundance of excellent cured forage in the Cypress Hills and Rocky Mountain Foothills. Seed stalks are usually produced very sparsely so basal leaves normally account for approximately 95 per cent or more of the dry weight of the plant. The cured leaves deteriorate very slowly and may be found in a good state of preservation up to 12 months or more after curing occurs.

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CRESTED
WHEATGRASS

Fig. 3

Crested wheatgrass
Agropyron cristatum
(cultivated)

This is an introduced grass from the arid steppes of Russia and western Siberia. Because of its drought resistance, winter hardiness, and ability to compete successfully with most weeds, it has been used very extensively in regrassing programs in the dryland areas of the Prairie Provinces, (6). According to Kirk and Stevenson (32), it produces very palatable and highly nutritious forage for either hay or pasture; even the straw of crested wheatgrass, which has been harvested for seed, is readily eaten. Nevertheless, it is considered inferior to speargrass or fescue as a stem-cured forage. Many of the stem leaves break off during the fall and early winter but the stems resist weathering quite well and constitute most of the cured forage, Table 1.

Fig. 4

12" Common or Smooth brome grass

Bromus inermis

(cultivated)

Brome is also an introduced grass, being native to Europe, Siberia, and China. It is not as winter hardy or as drought resistant as crested wheatgrass, although it stands more drought than most of our cultivated grasses (30). For this reason it is not recommended for the dryland areas of the West, but it is used considerably for regrassing where moisture conditions are more favorable, such as occur in the Cypress Hills area (6). Where it is well adapted to the climate, it is known as an outstanding producer of excellent hay and pasture. As a stem cured forage, it is considered to be of very little nutritional value to the range animal and distinctly inferior to the three grasses previously discussed. During the early fall and winter months, under Swift Current conditions, practically all of the stem leaves and some of the stems, of the mature plants, break off and blow away.

W. H. H.

Common to most of the islands

Islands of the Pacific

(continued)

Bromus is also an introduced grass, being native to Europe, Africa, and China. It is not as winter hardy as some of the other grasses, although it stands up to frost better than most of our cultivated grasses (30). For this reason it is not recommended for the island areas of the West, but it is used considerably for repressing where pasture conditions are more favorable, such as occur in the Oahu area (31). There it is well adapted to the climate, it is known as a hardy grass, and produces of excellent hay and pasture. As a stem cover crop, it is considered to be of very little nutritional value to the range animal and distasteful to the horse (32). During the early fall and winter months, when the ground is covered with snow, practically all of the stems and leaves are eaten, of the stems, of the mature plants, pressed and dried away.

Sites   (Location and Description)

All sites were located within the mixed prairie area in the Swift Current district. Each one occupied about three-quarters of an acre.

1. Speargrass

This site was located on the east side of a low sand dune within an experimental exclosure of the Webb Research Area, 16 miles west of Swift Current. Sub-dominant grasses on this area were sandgrass, Calamovilfa longifolia (Hook) Scribn., and Indian ricegrass, Oryzopsis hymenoides (R. and S.) Ricker., with a few shrubs, wolf willow, Elaeagnus commutata Bernk., and chokecherry Prunus melanocarpa (A. Nels) Rydb., scattered over the lower third of the area. A few other grasses and forbes of minor importance were also found within the area.

2. Fescue

The fescue site was located on a fairly steep northern slope, within the Alexander Coulee Research Area, approximately six miles east of Swift Current. The slope was characterized by almost a pure stand of fescue - all other species being distinctly of minor importance - surrounded by a typical mixed prairie association.

Station (L-100) and Station (L-101)

All sites were located within the same general area in the Swift Creek district. Most are located about 1000 feet from the river.

I. Station (L-100)

This site was located on the east side of a low sand dune within an experimental enclosure of the Swift Creek area. It lies west of Swift Creek. The dominant species on this site were sandhogs, *Salicaria* (leaf), and Indian ricegrass, *Oryzopsis hymenoides* (L. and G.) Hitchc. With a few shrubs, wolf willow, *Salix lasiolepis* (L.) Wats. and chokecherry, *Prunus virginiana* (L.) Mill. (L-100) were scattered over the lower third of the area. A few other species and species of minor importance were also found within the area.

II. Station (L-101)

The second site was located on a fairly steep sand dune slope, within the Alexander County Research Area, approximately 1000 feet east of Swift Creek. The slope was characterized by almost a pure stand of fescue - all other species being of minor importance - surrounded by a typical desert prairie association.

3. Crested wheatgrass

The crested wheatgrass site was located on a level area in the south-west corner of the Swift Current airfield, approximately four miles east of Swift Current. Other grasses on the area were western wheatgrass, Agropyron Smithii Rydb., and wild barley, Hordeum jubatum L.

4. Brome

The brome grass site was located in the south-west corner of a pasture field at the intersection of highways 1 and 4, within the boundaries of the Experimental Station proper. There was a relatively pure stand of brome grass with some crested wheatgrass and a few other Agropyrons scattered over the sampling area.

Sampling Methods

1. Establishment of Quadrats

To assure that a representative sample was obtained from each site, two base lines were established at right angles to each other along two sides of each experimental area. The site was then mapped out on paper and divided into numbered

3. Created wetlands

The created wetlands site was located on a level area in the south-west corner of the Swift Creek Wildlife Refuge. Approximately four miles east of Swift Creek, on the area were western wheatears, Agropyron smithii Rydb., and wild barley, Hordeum junceum L.

4. Brome

The brome site was located in the south-west corner of a pasture field at the intersection of Highway 1 and 1/2 mile in the boundaries of the Experimental Station proper. There was a relatively pure stand of brome with some created wetlands and a few other Agropyron scattered over the sampling area.

Sampling Methods

1. Establishment of quadrats

To ensure that a representative sample was obtained from each site, two base lines were established at right angles to each other along two sides of each experimental area. The site was then mapped out on paper and divided into numbered

square meter units. Twelve of these meter quadrats were selected at random. Each quadrat was established within the area by measuring inward from the two base lines; the corners were marked with spikes. The quadrats were again sub-divided into quarters, a different quarter being cut at each stage of growth. Not all of the quarter quadrats contained the species being sampled but never less than eight samples were obtained from each site.

Note: Owing to the extremely dry weather, there was little early spring growth on the native grass sites. To obtain a large enough sample at Stage 1, fescue and speargrass were sampled anywhere within the experimental area.

2. Growth Stages

In order to study changes which occur as the plant develops and matures - information which might be essential for the interpretation of the data from the cured stage - the grasses were sampled at several stages of growth. Growth stages were preferred to definite dates so that proper comparisons could be made between species as the season progressed.

square meter units. Twelve of these units, marked with self-
colored at random. Each quadrat was subdivided into four equal
by measuring lines from the two outer lines; the corners were
marked with spikes. The quadrats were again subdivided into
quarters, a different quarter being cut at each stage of sub-
Not all of the quarter quadrats contained the species being stud-
ied but never less than eight samples were obtained from each
site.

Note: Owing to the extremely dry weather, there was little
early spring growth on the native grass areas. No
obtain a large enough sample at stage 1, forests and
spruce-fir were sampled anywhere within the experimental
area.

2. Growth Stages

In order to study changes which occur in the plants
developed and mature - information which might be essential
for the interpretation of the data from the first stage - the
samples were collected at several stages of growth. Growth
stages were preferred to relative dates as they are more
reliable and could be made between species and the season progressed.

The five stages employed by Patton (50) for lignin and cellulose studies in Montana were chosen. They are as follows:

- (1) Early leaf growth.
- (2) Flowering culms in full bloom.
- (3) Seeds ripe.
- (4) Cured (most of the seeds fallen, plants dried on the stem).
- (5) Remnants of the previous year's growth, (sampled in early spring). The fifth growth stage was considered relatively unimportant, so only a few analyses and tests were carried out for special purposes.

Hereafter, growth stages will be referred to by number, or as cured (Stage 4).

3. Harvesting

Because of diurnal variations known to exist in the chemical composition of plants (44), the grasses were sampled in the afternoon, usually between 2:00 p.m. and 5:00 p.m. They were cut with shears, approximately one inch above the crown, except for fescue which was cut at the top of the rough basal growth. The old growth was separated from the current year's

The five stages of growth are:

and collected samples in the same order. They are as

follows:

- (1) Early leaf growth.
- (2) Flowering culms in full bloom.
- (3) Seeds ripe.
- (4) Cured (most of the seeds fallen, plants dried on the stem).
- (5) Remnants of the previous year's growth, (sampled in early spring). The fifth growth stage was considered relatively unimportant, so only a few samples and tests were carried out for special purposes.

Hereafter, growth stages will be referred to by number, or as

cured (stage 4).

3. Harvesting

Because of diurnal variations there is a considerable variation in the chemical composition of plants (4), the reason was similar in the afternoon, usually between 2:00 p.m. and 5:00 p.m. They were cut with shears, approximately one inch above the crown, except for leaves which was cut at the top of the culm. The old growth was separated from the current year's growth.

and growth/discarded. Samples were divided into leaf and stem leaving the leaf sheath on the stem. Stem leaves of speargrass were included with basal leaves.

According to Gibbs (23) the leaf sheath of Blue panicum (Panicum antidotale) Retz., resembles the leaf more closely in chemical composition than it does the stem. This is probably true of other grasses as well. However, most of the leaves on the stems of brome and to a lesser extent of crested wheatgrass, break off and blow away after the plant dries in the early fall leaving a biological unit which consists mainly of stem and leaf sheaths. Conversely, the major part of the native grasses in the cured stage consists of basal leaf, Table 1. Thus a natural division exists which appears suitable for animal nutrition studies because most of the dry matter obtained by the grazing animal is considered in each case.

To make sure that the ripe seeds would not interfere with the qualitative starch test at Stage 3, the heads of the cultivated grasses were analysed separately. The speargrass heads are difficult to separate from the leaf sheath so they were left with the stem but the ripe seeds were removed. Residual seeds for all grasses at Stage 4 were removed for the same reason.

growing upwards. The leaves were divided into leaf and stem
leaving the leaf sheath on the stem. The leaves of grasses
were included with stem leaves.

According to Hodge (1943) the leaf sheath of the
perennial (*Pennisetum polystachion*) grass, resembles the leaf more
closely in chemical composition than it does the stem. This
is probably true of other grasses as well. However, most of
the leaves on the stems of grasses and to a lesser extent of
cereal grasses, break off and blow away after the plant
dries in the early fall leaving a stalk which is which consists
mainly of stem and leaf sheath. Conversely, the region part
of the native grasses in the United States consists of leaf and
stem. Thus a natural division exists in grasses which appears suitable
for animal nutrition studies because most of the dry matter con-
tained by the grazing animal is contained in each case.
To make sure that the ripe grasses were not intermixed
with the qualitative studies, leaf at Stage 3, the heads of the
cultivated grasses were weighed separately. The grasses
heads are difficult to separate from the leaf sheath as they
were left with the stem and the ripe heads were removed. The
heads were removed for all grasses at Stage 4 were removed for the
same reason.

The stem of fescue was not analysed at any stage because very few culms were found within the sampling area.

4. Size of Sample

The quantity of forage dried for chemical analysis was limited to approximately 500 grams (fresh weight), by the size of the drying units. Accordingly, in some cases, the sample from the individual quarter quadrat was re-sampled to obtain a smaller unit, which was included in a composite sample for drying.

Drying the Samples

Lignin and soluble carbohydrate determinations were believed to be of fundamental importance when this investigation was started.

MacDougall and DeLong (37) showed that initial drying temperature had a direct influence on the apparent lignin content of plant materials; higher drying temperatures - within

The area of forest was not surveyed in 1950 because very few of the trees were large enough to be measured.

4. Size of sample

The quantity of sample used for each of the 10 plots was limited to approximately 100 trees (1000 kg) of each size of the living units. In some cases, the sample was taken from the individual trees that were regarded to be the most typical, which was included in a separate sample for drying.

5. Sample size

It is not possible to calculate the sample size for the 10 plots because of the small number of trees in each plot. The sample size was therefore determined by the number of trees in each plot.

Macdonald and Macdonald (1957) showed that the sample size for the 10 plots was determined by the number of trees in each plot. The sample size was therefore determined by the number of trees in each plot.

the range studied - gave increased values for lignin. Furthermore certain carbohydrates readily change from one form to another when plant material is heat-dried and caramelization may occur at temperatures high enough to inactivate enzymes. Obviously then, drying by heat was undesirable.

Ellis et al (20) recommended air drying for plant materials where subsequent lignin determinations are to be made. However, it is well known that plants allowed to dry slowly, lose dry weight because of the cell respiratory activities. Dexter (14) showed that alfalfa hay air-dried in the mow over a period of three weeks lost a considerable portion of its sugar although whether these losses were caused by respiration or transformations was not known. While the error in lignin determinations caused by this factor would be small, the loss of sugars could introduce a rather large error into a soluble carbohydrate estimation. For this reason, air-drying was also considered unsatisfactory.

The lyophylization technique of Davies et al (12) is claimed to be capable of removing moisture from small and medium sized forage samples with minimal changes occurring in chemical composition. It was selected as the most suitable method available for drying the grass samples.

A brief general description of the method used by Davies and co-workers with slight modifications in equipment is as follows:

A closed insulated container enclosing a smaller unit, which was surrounded by powdered dry ice (solid CO_2), was transported to the sampling site. As the grass was harvested it was dropped into the inner container where it was rapidly frozen. Both containers were now closed, and in this state the sample would remain frozen for several hours or days, depending on the quantity of dry ice, efficiency of insulation, air temperatures, and other factors.

For the present studies the Kilner jar and Thermos flask used by Davies et al (12) was replaced by a one gallon insulated ice cream pack (available from dairy supply houses). The inner container was merely a tall metal can, the type sometimes used for packing and shipping hydrogen peroxide. When placed inside the ice cream pack there was ample room around it for a charge of dry ice.

The sample was taken to the laboratory, and transferred while still frozen, to a large, round-bottomed flask (5 liter). The flask was attached at once to a high vacuum system, and evacuated by an efficient oil pump. The vacuum unit consisted essentially of a cooled condenser immersed in a dry ice acetone bath at approximately $-78^\circ\text{C}.$, with a vacuum

A brief general description of the apparatus used is

as follows:

A closed insulated container containing a smaller flask which was surrounded by powdered dry ice (solid CO₂), was immersed in the sampling site. As the flask was immersed it was dropped into the inner container where it was rapidly frozen. Both containers were now closed, and in this state the sample would remain frozen for several hours or days, depending on the quantity of dry ice, efficiency of insulation, air temperature, and other factors.

For the present studies the inner jar and flask were used. The flask used by Davies et al (11) was replaced by a one gallon insulated ice cream pail (available from dry supply houses). The inner container was merely a tall metal can, the type sometimes used for packing and shipping hydrogen peroxide. Inside the ice cream pail there was space for a charge of dry ice.

The sample was taken to the laboratory, and then removed while still frozen, to a large, round-bottomed flask (5 liter). The flask was attached at once to a high vacuum system, and evacuated by an efficient oil pump. The vacuum unit consisted essentially of a cooled condenser immersed in dry ice acetone bath at approximately -75°C., with a vacuum

lead-off to the pump through the condenser.

The Thermos flask used as a container for the dry ice acetone mixture which chilled the condenser was replaced by an ordinary one-half gallon can insulated on the outside with a layer of shredded paper 2 - 3 inches thick. While the Thermos flasks used by the authors would undoubtedly be more economical in utilization of dry ice, the two alternates described above served the purpose quite well.

Under vacuum the ice is sublimed out of the frozen plant tissues and refrozen on the inside of the condenser. The forage remains frozen until dry, a period of 5 - 6 hours being required to reduce the moisture content to approximately 0.5 per cent (12). By substituting a charge of phosphorus pentoxide for the condenser, the moisture content can be further reduced if so desired.

Difficulties encountered while using the freeze dry method were mainly concerned with insufficient vacuum. A Cenco Hyvac pump in good condition, rated by the manufacturer as capable of developing a vacuum of 0.0004 m.m. mercury, was found to be satisfactory. Several other pumps, either with a lower potential vacuum or else in poor condition, gave very disappointing results.

lead-off to the pump through the condenser.

The thoron flask used as a condenser for the dry

ice acetone mixture which chilled the condenser was replaced

by an ordinary one - this can be fitted on the outside

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Under vacuum the ice is sublimed out of the thoron

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Difficulties encountered while using the freeze

dry method were mainly concerned with the handling of vacuum,

since it was very in good condition, raised by the condenser

as capable of developing a vacuum of 0.0001 mm. mercury, and

found to be satisfactory. Several other pumps, either with a

lower potential vacuum or else in poor condition, gave very

disappointing results.

It was also found that the thick brome stems took about twice as long to dry as did leafy material, in some cases up to 12 hours was required. A method of shredding thick material before freezing it would speed up drying operations considerably.

Experience gained while using the freeze dry method indicates that it is quite suitable for drying a small number of samples for specialized research purposes. In its present form it appears to be too time- and labor-consuming for routine procedures where large numbers of samples are involved.

Grinding and Storing

The samples were ground in a small Gallenkamp hammer mill, so that 95 per cent would pass through an 80 mesh screen. They were stored in air-tight containers, (half pint sealers with rubber rings).

Because of the difficulties involved in keeping the ground material at a moisture free basis while grinding and carrying out the chemical analyses over an extended period, it

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samples for specialized research purposes. In the present form
it appears to be too time- and labor-consuming for routine pro-
cedures where large numbers of samples are involved.

Grinding and Storing

The samples were ground in a small (1 liter) mortar
mill, so that 95 per cent would pass through an 80 mesh screen.
They were stored in air-tight containers, (half pint bottles
with rubber rings).

Because of the difficulties involved in grinding the
ground material at a constant rate while grinding and
carrying out the chemical analyses over an extended period, it

was allowed to return to a moisture content approaching that of air-dried material. Moisture content was determined periodically but there was little change from these values. According to the observations of Ekelund (18) there should be very little storage losses at this moisture content.

Biochemical Determinations

1. Lignin

The 72 per cent H_2SO_4 method of Ellis et al (20) was adopted and used as published. This method has proved useful and is being used quite extensively in nutritional work. Recent studies sponsored by the A.O.A.C. (19) indicate that determinations made using this method show a reasonably satisfactory degree of reproductibility both within and between laboratories.

2. Cellulose

The choice of a suitable method was difficult as there is no general agreement on a satisfactory method for nutritional

was allowed to remain in a moisture cabinet for 24 hours of air-dried material. The material was then placed in a desiccator but there was little change in weight. According to the observations of Kjelung (10) there should be very little weight losses at this moisture content.

Biological Determinations

1. Lipids

The 75 per cent $CHCl_3$ method of Ellis et al (11) was adopted and used as published. This method has proved useful and is being used quite extensively in nutritional work. Recent studies sponsored by the A.O.A.C. (12) indicate that the methods made using this method show a reasonably satisfactory degree of reproducibility both within and between laboratories.

2. Cellulose

The choice of a suitable method was difficult in these cases as no general agreement on a standard method for nutritional

studies. This situation is well illustrated by Hallsworth's (28) critical comparison of seven different methods for cellulose determinations on feedstuffs. He found that the cellulose obtained by any one method varied widely in both amount and composition of the isolated product.

The modified Norman Jenkins method developed by Matrone et al (39) has been used frequently in nutritional studies since 1946. It is claimed to have an advantage over most methods for nutritional studies in that it isolates a biological unit which includes not only the cellulose, but also much of the so called cellulosans (pentosans and other materials associated with the "true" cellulose in the cell wall), substances which appear to be similar in degree of digestibility to the hexosan cellulose (21). This appears to be an advantage for nutritional work. Unfortunately however, studies sponsored by the A.O.A.C. (19) have indicated that this method is unsatisfactory in its present form.

Histological observations early in this investigation indicated that a considerable part of the native grass leaf consisted of cellulose fibres presumably in a relatively pure form. A method that would isolate primarily hexosan cellulose and thus supply a quantitative estimate of the amounts present seemed desirable. The Dichromate method of Druce and Willcox (16) appeared to fit this criterion most closely so it was used. It

has the admitted disadvantage of being so recent that insofar as the writer is aware, no data other than that supplied by the authors (17) are available to check their results.

The method was modified slightly. Filter sticks were substituted for the poplin Buchner funnel method of filtration and Gooch crucibles with asbestos mat were used for ashing instead of the silica crucibles specified by the authors. Use of the filter sticks permitted all filtering and washing operations to be carried out in one beaker, thus eliminating transfers.

3. Ether Extract (Crude fat)

Crude fat analyses were carried out by the official A.O.A.C. (43) procedure. Since most feed laboratories now substitute petroleum ether for ethyl ether as the extracting solvent, the method was modified to this extent.

4. Protein

The standard A.O.A.C. (43) Kjeldahl method for determining protein (total nitrogen x 6.25) was used, with the addition of one-quarter part of selenium in addition to the regular salt

mixture. The Boric acid modification of Scales and Harrison (56) was used in the distillation procedure.

5. Starch

A qualitative method was used to check the cured forage and determine whether starch was present or not. The method used by Ekelund (18) to test Swedish hays for starch appeared to be particularly well suited for this purpose, so it was used. Since the method is very simple and requires little space to describe it is outlined here.

Duplicate one-gram samples of ground forage were weighed out and placed in two large test tubes. Ten mgm. of wheat flour were added to one for a check. Both samples were boiled in a water bath for two minutes, cooled and 0.5 ml. solution of iodine (2 gm. I_2 + 6 gm. KI in 100 ml. water) was added. If the intensity of the color of the test solution did not approach or exceed that of the check it was considered that there was less than 1% of starch present in the sample.

mineral. The results of the analysis of the mineral
(2) was used in the classification of minerals.

2. Discussion

A qualitative analysis was used to identify the mineral-

age and determine whether it was a mineral or not. The

method used by the author (1) to test whether a mineral is a mineral

appeared to be particularly well suited for this purpose, as

it was used, since the method is very simple and requires little

space to describe it in detail.

The following are the results of the analysis of the mineral

which was found in the large rock mass. The results of the

analysis were used to identify the mineral. The results of the

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Histological Techniques

1. General

A typical leaf and stem were selected for sectioning from each quadrat on every sampling site, at all growth stages. Leaves, whether basal or stem, were sectioned at a point halfway between leaf tip and leaf base. In the case of stem leaves the second leaf from the top of the stem was used. Stems, including the leaf sheaths, were sectioned at a point approximately halfway between the two uppermost nodes. Rough fescue has no stem nodes so it was sectioned halfway between panicle and stem base.

A free hand method of sectioning was chosen so that microchemical tests could be applied to fresh tissue.

2. Microchemical Tests

(1) Lignin

The hydrochloric acid, phloroglucin method of Johansson (31) was applied to cross-sections of leaf or stem at all stages of growth.

(2) Fat

The Sudan IV method as outlined by Johansson (31) was used on cross-sections of the native leaves at Stages 4 and 5.

Microchemical Tests

1. General

A typical leaf and stem were selected for sectioning from each quadrat on every sampling site, at all growth stages. Leaves, whether basal or stem, were sectioned at a point halfway between leaf tip and leaf base. In the case of stem leaves the second leaf from the top of the stem was used. Stems, including the leaf axils, were sectioned at a point approximately halfway between the two youngest nodes. Young leaves near the stem nodes so it was sectioned halfway between petiole and stem base. A free hand method of sectioning was chosen so that microchemical tests could be applied to fresh tissues.

2. Microchemical Tests

(1) Aluminum

The hydrochloric acid, aluminum method of Johnson (1951) was applied to cross-sections of leaf or stem at all stages of growth.

(2) Calcium

The method outlined by Johnson (1951) was used on cross-sections of the native leaves at stages 4 and 5.

3. Sectioning Technique

Twelve pieces of leaf or stem to be sectioned, were laid out in order on the table. Freehand sections were cut from each with a sharp razor blade, while holding the material between two pieces of pith, keeping both blade and pith saturated with 70 per cent ethyl alcohol. The sections were dropped into a dish containing 70 per cent ethyl alcohol and left from 30 - 60 minutes, to dissolve out the chlorophyll. This last step was found to be essential to obtain a sharp, clear differentiation of tissues when the lignin test was applied.

4. Staining

After the chlorophyll had been removed, the sections were transferred to a slide and stained according to the particular test desired.

5. Photomicrography

After the microchemical test had been applied, the sections were examined under low power using a small student model microscope. Several of the most uniform and representative of the group were changed to a clean slide, mounted in a

2. Description of the material

Twelve pieces of fabric, each of which was

folded over in order to form a double.

From each piece a small square was cut, and the material

between the pieces of fabric, which was also cut out.

With the exception of the small squares, the material was

all contained in the small squares, and the material was

all contained in the small squares, and the material was

all contained in the small squares, and the material was

all contained in the small squares, and the material was

3. Results

After the material had been removed, the results

were found to be as follows, and the results were

as follows.

4. Discussion

From the experimental results it can be seen

that the material was not removed, and the results

were found to be as follows, and the results were

as follows.

glycerol water solution and photomicrographed.

The use of the extra microscope for the preliminary examination of the slides was found to be very convenient, as it permitted the eyepiece camera to be left attached to the research microscope in readiness for the photographic step. This is especially desirable when testing for lignin as the phloroglucin stain is only temporary and fades noticeably within a few minutes.

Very few of the sections tested for fat were photographed as in most cases the fat globules showed up equally well or better in those sections stained for lignin and more contrast was obtained in the photomicrograph.

RESULTS

On the basis of growth habit i.e. ratio of leaf to stem and the anatomical structure, the grasses fall into two fairly distinct groups. Table 1 shows that 95 per cent or more of the dry weight of the native grasses at Stage 4 consisted of leaves. Conversely approximately 90 per cent of the dry weights of the cultivated grasses at Stage 4 consisted of stems. Therefore the most important comparisons will be made between native grass leaves on the one hand and cultivated grass stems on the other.

Table 1

Relative Proportions of Leaf to Stem
in Percentage of Dry Weight

Growth stage	Grass	Leaf	Stem	Head
2	Speargrass	91.0	9.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	15.0	85.0	
	Brome	24.0	76.0	
3	Speargrass	93.0	7.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	16.0	66.0	18.0
	Brome	15.0	65.0	20.0
4	Speargrass	96.0	4.0	
	Fescue*	98.0	2.0	
	Crested wheatgrass	13.0	87.0	
	Brome	9.0	91.0	

* Fescue not weighed, estimated only.

With the exception of Stage 5 the results of the analyses of leaf stems and heads reported in Tables 2, 3, 4, and 5, were calculated to a whole plant basis and are reported in Fig. 5, 25, 30, and 31 respectively.

Lignin

The important role that lignin plays in the digestibility of forages has been discussed. Examination of Fig. 8, 14, and 15 shows that lignified tissue in the immature native grass leaf is confined chiefly, if not entirely, to the vascular bundles. Furthermore, in the fescue leaf, Fig. 13, these bundles are smaller in size, and less numerous than they are in the speargrass leaf, Fig. 6, thus constituting a smaller proportion of the total cross sectional leaf area. In addition, Fig. 9 and 16 indicate that as the season progresses, lignin apparently does not infiltrate and encrust the adjacent cellulose fibres to any great extent. Neither is there any sign of lignification in the walls of the mesophyll cells. Biochemical analyses corroborate these observations, the initial lignin content of fescue being much lower than that of speargrass, Fig. 5, and the slope of both curves being similar and relatively flat, indicating that no large increase in lignin content occurred during the season.

Fig. 5

Effect of Growth Stage on Lignin Content

The two cultivated grasses consist entirely of leaf and stem bases at Stage 1. Fig. 17 and 21 show that here again the major portion of the lignin is confined to the wide-spaced vascular bundles. However, as the plant reaches a more mature stage, there occurs a reversal of the ratio of leaf to stem and there is a preponderance of stem which becomes more marked as the season advances, Table 1.

In the early stages of stem growth, Stage 2, Fig. 19 and 23, the lignification of cell walls was mostly confined to the vascular bundles. At a later growth stage, Stage 4, Fig. 20 and 24, heavy lignification occurred in the walls of the cellulose fibres and the parenchyma adjacent to the vascular bundles. A very high proportion of the cross sectional area of the stem tissues was now lignified.

The lignin determinations reflect this relationship. Fig. 5 shows that a very low lignin concentration was present in both cultivated grasses, Stage 1. With advancing maturity a **steady** rise in percentage took place and the steep slope of both curves indicated a similar trend for the brome and crested wheatgrass.

Fig. 10 shows that the stem and leaf sheath of spear-grass consisted chiefly of structural material. At Stage 2 there was little lignification of the fibres and parenchyma.

The two cultivated species showed a similar pattern and stem bases at Stage I, II and III show that there was the major portion of the lignin is confined to the wide-spaced vascular bundles. However, as the plant reaches a more mature stage, there occurs a reversal of the ratio of I to II and there is a preponderance of stem lignin. This pattern was marked as the season advanced, Table I.

In the early stages of stem growth, Stage I, II and III, the lignification of cell walls was mostly confined to the vascular bundles. In a later growth stage, Stage IV, Fig. 40 and 41, heavy lignification occurred in the walls of the cellulose fibres and the parenchyma adjacent to the vascular bundles. A very high proportion of the cross sectional area of the stem tissues was now lignified.

The lignin concentration followed this relationship. Fig. 5 shows that a very low lignin concentration was present in both cultivated species, Stage I. With increasing maturity a steady rise in percentage lignin was observed and the lignin content both curves indicated a similar trend for the two species, wheatgrass.

Fig. 10 shows that the stem and leaf bases of wheatgrass consisted chiefly of suberized material. At Stage I there was little lignification of the stems and branches.

At Stage 4 (not illustrated) the fibres and parenchyma were both heavily lignified. This agrees with the high lignin values reported for speargrass stem at Stage 4, Table 2.

The histological observation on the progress of lignification in the cultivated grasses are similar to those reported by Drapala et al (15) for red clover.

At stage 4 (not illustrated) the lines are somewhat more
heavily lighted. This stage is the 10th
value reported for open water at stage 4, Table 2.
The lithological observation of the progress of the
diffusion in the oil field, however, was similar to those re-
ported by Phelps at 21 (12) for red clover.

Common speargrass

Fig. 6 (x 40)

Fig. 7 (x 20)

Fig. 8 (x 80)

Fig. 9 (x 80)

Cross Sections of Leaf and Stem

Fig. 6 (Stage 4). General outline of rolled leaf.

Fig. 7 (Stage 2). General outline of stem and leaf sheath.

Fig. 8 (Stage 2). Basal leaf. Fibre development not yet complete. No lignification of fibres.

Fig. 9 (Stage 4). Basal leaf. Fibre development complete; some lignification in fibres especially below vascular bundles. Fat globules visible in mesophyll cells.

Common speargrass

Fig. 10 (x 80)

Fig. 11 (x 80)

Fig. 12 (x 80)

Cross Sections of Leaf and Stem

Fig. 10 (Stage 2). Detail of stem and leaf sheath.

Fig. 12 (Stage 3). Basal leaf showing disintegration of the mesophyll; general form and structure of the leaf maintained by supporting fibres.

Fig. 11 (Stage 2). Stem leaf similar to basal leaf but without band of fibres inside lower epidermis.

Rough fescue

Fig. 13 (x 40)

Fig. 14 (x 80)

Fig. 15 (x 80)

Fig. 16 (x 80)

Figs. 13 to 16. Cross sections of basal leaves showing stages of fibre development and progressive lignification.

Fig. 13 (Stage 1). General outline of permanently rolled leaf.

Fig. 14 (Stage 1). Early fibre development, no lignification.

Fig. 15 (Stage 2). Further fibre development, little or no evidence of lignification.

Fig. 16 (Stage 4). Complete fibre development, slight lignification. Fat globules clearly visible in mesophyll cells.

Crested wheatgrass

Fig. 17 (x 40)

Fig. 18 (x 20)

Fig. 19 (x 80)

Fig. 20 (x 30)

Cross Sections of Leaf and Stem

Fig. 17 (Stage 1). General outline of a narrow leaf showing leaf structure which is intermediate between brome and native grasses.

Fig. 18 (Stage 2). General outline of stem and leaf sheath.

Fig. 19 (Stage 2). Stem and leaf sheath. Slight lignification of fibres and of stem parenchyma adjacent to the bundles.

Fig. 20 (Stage 4). Stem and leaf sheath. Much heavier lignification of both fibres and stem parenchyma.

Common brome

Fig. 21 (x 20)

Fig. 22 (x 80)

Fig. 23 (x 80)

Fig. 24 (x 80)

Cross Sections of Leaf and Stem

Fig. 21 (Stage 1). General outline of a narrow leaf.

Fig. 22 (Stage 1). Enlarged view of leaf margin, Fig. 21, showing widely spaced bundles, few fibres and thin-walled epidermal cells.

Fig. 23 (Stage 2). Stem and leaf sheath showing little or no lignification of fibres but slight lignification of stem parenchyma.

Fig. 24 (Stage 4). Stem and leaf sheath showing much heavier lignification at both fibres and stem parenchyma and disintegration of mesophyll in the leaf sheath.

Table 2

Lignin Content of Four Grasses in Percentage of Dry Weight
(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	8.3	4.5	5.5	4.6
	2	9.3	5.4	6.3	6.2
	3	10.0	5.3	8.0	7.9
	4	10.1	6.3	8.6	10.5
	5	9.8	6.9	-	-
Stem	2	9.5	-	9.2	8.8
	3	11.4	-	8.7	9.5
	4	12.0	-	10.9	12.1
Head	3	-	-	9.4	7.8

Table 2

Light Content of Four Masses in Percentage of Dry Weight
(Average of Two Determinations)

Stage	Native	Cultivated	From
1	8.3	2.2	4.6
2	9.3	6.3	6.3
3	10.0	8.0	7.9
4	10.1	8.0	10.5
5	9.6	-	-
6	9.2	6.2	8.8
7	11.4	8.1	9.5
8	12.0	10.3	12.1
9	-	9.2	7.0

Cellulose

The success of the curing habit depends basically on the inherent ability of the aerial parts of the plant to resist deterioration over a period of several months following the curing process. The anatomy of the basal leaf of the native grass, Fig. 8 and 15, suggests that reinforcing cellulose fibres play a major role in the observed ability of these leaves to retain their physical form after curing occurs. These fibres form, in most cases, a complete band several fibres wide, around the inside of the lower epidermis, fescue having a heavier band than speargrass. In addition, the vascular bundles are strongly reinforced by fibres extending above and below in the form of "I" beams. The total cross sectional area occupied by the fibres appearsto be somewhat greater in the case of fescue than in that of speargrass, Fig. 9 and 16. This factor agrees with the slightly higher cellulose values reported for fescue leaf than those for speargrass leaf, Fig. 25.

An entirely different picture is presented by the anatomy of the introduced grass leaf. These leaves, brome in particular, Fig. 17 and 21, are wide, thin, less compact, with only a few reinforcing cellulose strands occurring immediately above and below the widely separated vascular bundles, and at the leaf edge, Fig. 21. The weak thin-walled epidermal cells

Fig. 25

Effect of Growth Stage on Cellulose Content

of the brome leaf, Fig. 22, should also be observed. Hence the brome leaves are weakly reinforced and this explains why they break up readily soon after they mature in the fall, frequently as early as mid-September. Crested wheatgrass leaves do not lacerate and shred like the brome leaves and this is believed to be due to the fact that they are somewhat narrower, thicker and more compact, Fig. 17. They are much weaker structurally than the native grass leaves, however, and many of these break off after curing takes place. Part of the loss of crested wheatgrass leaves is frequently attributable to grasshopper damage, as well.

Fig. 12 shows that speargrass leaves retain their general form even after long periods of weathering, apparently due to reinforcing fibres.

Additional information on the importance of structural fibres in the leaf was obtained from the two sub-dominants on the speargrass site, namely, sandgrass and ricegrass. Sections were made of both grasses including the early growth, Fig. 26 and 28 and the previous year's growth, Fig. 27 and 29 still standing on the stem.

Fig. 26 shows that sandgrass has a wide, flat leaf similar in outward appearance to the brome leaf. The internal structure, however, is very different in that it has very closely

of the first leaf, fig. 25, which also is observed. Hence
the first leaf is usually produced and this explains why
they break so readily soon after they appear in the fall, fre-
quently as early as mid-September. In the case of leaves
do not increase and stand like the other leaves and this is
believed to be due to the fact that they are somewhat narrower,
thicker and more compact, fig. 17. They are much wider
than the other leaves, however, and many of
them break off when they are in place. Part of the loss of
created and leaves is the result of a tendency to break-
up, as well.
fig. 18 shows that the leaves are not only
and from very long periods of withering, apparently due
to withering leaves.
A list of information on the importance of structure
in the leaf was obtained from the two main divisions on
the opposite side, namely, secondary and tertiary. The
were made of some material including the early growth, fig. 26
and 27 and the previous year's growth, fig. 27 and 28 still
standing on the stem.
fig. 26 shows that secondary has a wide, flat leaf
leaf in which appearance to the stem leaf. The internal
structure, however, is very different in that it has very closely

Sandgrass

Fig. 26 (x 20)

Fig. 27 (x 80)

Indian ricegrass

Fig. 28, (x 80)

Fig. 29 (x 80)

Cross Sections of Stem Leaves

- Fig. 26 (Stage 2). General outline of wide thin leaf showing closely spaced vascular bundles.
- Fig. 27 (Stage 5). General form and structure of the leaf maintained by close arrangement of bundles and heavy reinforcement of fibres.
- Fig. 28 (Stage 2). Showing leaf structure similar to speargrass, Fig. 12.
- Fig. 29 (Stage 5). Showing disintegration of mesophyll but general form and structure maintained by heavy reinforcement of fibres.

spaced vascular bundles which are well reinforced above and below with strands of strong cellulose fibres, Fig. 27, in contrast to the widely spaced, poorly reinforced bundles of the brome leaf, Fig. 21. Although the soft internal tissues of the sandgrass leaf are obviously breaking up at Stage 5 outwardly the old leaf appears to be in an excellent state of preservation with general structure and form well maintained.

It should be mentioned here that this grass is of little value for pasture as it is very tough and unpalatable to livestock. Nevertheless it serves to illustrate the importance of leaf structure in relation to the curing process.

The ricegrass is very closely related to the speargrass (55) and the leaf structures are similar Fig. 11 and 28. The old leaf, Stage 5, Fig. 29, shows that the vascular bundles and reinforcing fibres are essentially intact, enabling the leaf to retain its physical form although the softer tissues have largely degenerated. This grass is regarded as a palatable, nutritious forage but it is of little economic importance in the area because of its relative scarcity.

Fig. 25 shows that a much higher proportion of the total cellulose was present in the native grass leaves at early growth stages than was the case for the cultivated grasses. These results are in agreement with the histological observations.

Table 3

Cellulose Content of Four Grasses in Percentage of Dry Weight
(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	22.7	23.0	18.8	16.4
	2	23.6	25.5	16.2	15.2
	3	24.2	26.8	18.1	18.1
	4	24.5	26.2	21.2	19.2
Stem	2	22.0	-	20.2	24.1
	3	24.5	-	24.1	26.5
	4	30.2	-	23.4	26.2
Head	3	-	-	10.6	11.7

Table 2

Cellulose content of four grasses in relation to dry weight
(average of two determinations)

Grass	Native		Domesticated	
	Great Plains	Florida	Great Plains	Florida
1	22.7	22.0	18.8	18.1
2	27.6	27.2	16.2	15.2
3	27.2	27.0	18.1	17.1
4	27.2	26.2	21.2	19.2
5	22.0	-	20.2	19.1
6	27.2	-	21.1	20.2
7	20.2	-	23.1	20.2
8	-	-	10.2	11.1

Ether Extract (Crude fat)

Fig. 30 indicates that the ether extract of both cultivated grasses followed the usual downward trend during the season, while that of speargrass remained nearly constant and that of fescue steadily increased.

Fig. 9 and 16 show that at least part of this increase is due to fat globules which reach substantial proportions particularly in the cells of fescue leaf, Fig. 16 and to a lesser degree in the speargrass leaf, Fig. 9.

As previously mentioned, most of the ether extracts from forages are of low nutritive value. In this instance, Stage 4, the ether extracts of the native grasses were not highly colored as was the case for earlier growth stages so that little chlorophyll was included. Furthermore, ether extracts of native grass leaves, Stage 5, Table 4, showed a relatively high value which shows that this characteristic was not confined to a single season. These observations suggest that possibly much of the crude fat obtained from the native forage, Stage 4, was actually made up of esters of fatty acids. These would be a small but valuable source of energy to the range animal, since true fats have two and one-quarter times the energy value of carbohydrates.

TABLE I (continued)

Fig. 10 indicates that the...
collected...
the season, while that of...
and that of... steadily increased.

Fig. 9 and 10 show that...
is due to the...
particularly in the cells of...
lesser degree in the...
Fig. 11.

As previously mentioned, most of the other extracts

from... are of low nutritive value. In this instance,

stage 1, the... extract of the... which was used

slightly colored as was the case for... growth stages in which

little chlorophyll was... in these cases, slight variations

of active... stage 2, stage 3, and stage 4, showing a...
high value which shows that the... and so -

... to a... These... and...
much of the... from... stage 2, stage 3, and stage 4,

was actually made up of... of... stage 2, stage 3, and stage 4,

small but valuable source of... stage 2, stage 3, and stage 4,

these... have... and... stage 2, stage 3, and stage 4,

...
...
...

Fig. 30

Effect of Growth Stage on Ether Extract

Table 4

Ether Extract Content of Four Grasses in Percentage of
Dry Weight

(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	3.5	3.6	3.7	3.1
	2	2.2	3.6	6.8	4.4
	3	3.9	4.5	5.4	4.5
	4	3.3	5.6	3.9	3.0
	5	4.6	3.5	-	-
Stem	2	3.2	-	3.1	1.9
	3	1.8	-	2.2	1.6
	4	1.1	-	1.2	0.1
Head	3	-	-	3.4	2.8

Table 4

Stem Weight Content of Four Species in Percentage of

Dry Weight

(Average of Two Collections)

Species	Growth Stage	Native		Cultivated	
		Species	Weight	Species	Weight
Horn	1	3.2	3.6	2.7	3.1
	2	2.2	3.6	2.8	4.4
	3	3.9	4.2	3.4	4.2
	4	3.3	2.6	3.0	3.0
	5	4.6	2.2	-	-
Stem	2	3.2	-	3.1	4.1
	3	1.6	-	2.2	1.6
	4	1.1	-	1.2	0.1
Horn	3	-	-	4.4	2.8

Protein

Fig. 31 shows that there was a steady decline in protein content of all four grasses throughout the season. Speargrass was noticeably higher in protein content than were the other three in the cured state, with crested wheatgrass intermediate and the other two very low. If protein is one of the limiting factors in the digestibility of these roughages, as it may be, then speargrass and crested wheatgrass are sufficiently above the other two to make some difference in nutritive value. Since these data cover only one site for one season, no definite conclusions can be drawn.

Starch

There was no evidence of appreciable amounts of starch at the cured stage in any of the four grasses. Speargrass leaf showed a slightly positive test, but even it did not approach the 1% point.

Protein

Fig. 31 shows that there was a steady decline in protein content of all four grasses throughout the season. Speargrass was noticeably higher in protein content than were the other three in the later stages. It was noted that in the intermediate and the other two very low. It is possible that of the limiting factors in the availability of these proteins, as it may be, that speargrass was not so much affected and would likely show the other two a more marked difference in relative value. Since these data cover only one site for one season, no definite conclusions can be drawn.

Minerals

There was no evidence of appreciable amounts of minerals in the crude stems in any of the four grasses. Speargrass leaf showed a slightly positive value, but even it did not approach the 1% point.

Fig. 31

Effect of Growth Stage on Crude Protein

Table 5

Protein Content of Four Grasses in Percentage of
Dry Weight
(Average of Two Determinations)

	Growth stage	Native		Cultivated	
		Speargrass	Fescue	Crested wheatgrass	Brome
Leaf	1	17.6	13.5	17.3	15.4
	2	16.2	9.9	15.2	17.1
	3	11.6	8.9	10.1	14.4
	4	8.8	3.6	6.8	6.4
	5	-	2.6	2.9	-
Stem	2	9.3	-	8.1	9.1
	3	4.9	-	7.0	6.0
	4	4.3	-	5.5	2.3
Head	3	-	-	11.2	13.6

Table 2

Protein Content of Four Groups in Percentage of

Live Weight

(Average of two determinations)

Group	Protein Content (%)	Live Weight		Protein Content (%)	
		Group 1	Group 2	Group 3	Group 4
1	17.5	17.5	17.5	17.5	17.5
2	16.5	16.5	16.5	16.5	16.5
3	11.5	11.5	11.5	11.5	11.5
4	8.8	8.8	8.8	8.8	8.8
5	-	-	-	-	-
6	9.3	9.3	9.3	9.3	9.3
7	14.5	14.5	14.5	14.5	14.5
8	11.5	11.5	11.5	11.5	11.5

DISCUSSION

Growth was very poor on the speargrass, fescue and crested wheatgrass sites during the 1949 season. The bromegrass site was located in a slightly more favorable position and growth on this area was nearly normal. In addition, some of the late fescue growth was frozen before curing occurred. Whether drought or frost affected the results of the chemical analyses - other than possible hastening lignification (51) - is not known.

The values reported for the lignin analyses of the two cultivated grasses, Stage 4, are considerably lower than those reported for the corresponding grasses in Montana (50, 51). Most of this difference in results is believed to be due to the fact that in that work the so-called formalin procedure for their lignin determination was used. This method was found to give higher apparent lignin values for mature plant material than did the 72 per cent sulphuric acid method (20).

The importance of lignification in the curing process is believed to be not so much total lignin content of the forage but rather the position and extent of the lignified tissues. A large proportion of the native grass leaf, Stage 4, consists of non-lignified mesophyll tissue. Both the cell walls

and cell contents would probably be readily digested by range animals. Also in general, the fibres were not heavily lignified and hence the cellulose in their walls should be quite susceptible to bacterial attack and decomposition in the digestive tract of herbivora.

Conversely, a very high proportion of the stem material of the cultivated grasses is structural material most of which is heavily lignified at Stage 4. This material would probably be digested to a lesser degree than that obtained from the native grass leaves and would provide the animal with less energy.

Further information in this respect is supplied by Patton's (50) observation that blue grama grass, one of the best of the cured native grasses, has a very high lignin content at an early vegetative stage, and a relatively high lignin content in the cured stage. Structurally the leaf of this grass is very similar to sandgrass (60) illustrated in Fig. 26. The numerous and closely-spaced vascular bundles would almost certainly result in a fairly high lignin content but apparently this lignin does not seriously interfere with the utilization of this grass by the range animal.

The crested wheatgrass stems usually stand weathering fairly well, but brome stems are not so resistant and a considerable number break off and blow away during the fall and early winter. This apparent weakness of brome stems may be due to two factors, namely:

and cell contents would probably be readily digested by ruminants. Also in general, the leaves tend to be heavily lignified and hence the cellulose in their walls would be quite unpalatable to beef and other animals and resistance to the digestive tract of herbivores.

Consequently, a very high proportion of the stem material of the cultivated grasses is not suitable for use as food for which is heavily lignified at stage 4. This material would probably be digested to a lesser degree than that obtained from the native grass leaves and would provide the animal with less energy.

Further information in this respect is supplied by Lutton's (50) observation that blue grass (Poa), one of the best of the cured native grasses, has a very high lignin content at an early vegetative stage, and a relatively high lignin content in the cured stage. Practically the leaf of this grass is very similar to sandgrass (60) illustrated in Fig. 50. The numerous and closely-spaced vascular bundles would almost certainly result in a fairly high lignin content but apparently this lignin does not seriously interfere with the utilization of this grass by the range animal.

The cured whorlgrass stems usually stand erect and fairly well, but some stems are not so resistant and a considerable number break off and blow away during the fall and early winter. This apparent weakness of some stems may be due to two factors, namely:

- (1) The hollow brome stem, Fig. 23, may be weaker than the solid or semi-solid crested wheatgrass stems, Fig. 19 and 20.
- (2) The brome stem may be more brittle than that of crested wheatgrass owing to a relatively high lignin content, Table 2. According to Phillips (52) a high lignin content makes plant tissues brittle but the exact concentration required to reach this point is not stated.

The lignin values obtained for speargrass and fescue leaves, Stage 5, are worthy of special comment. They are almost identical with those reported for Stage 4, Table 2. Unless the initial lignin content of these grasses in 1948 was much lower than that of 1949, little losses of soluble constituents have occurred, otherwise the percentage of lignin would have increased.

The results from the dichromate method (16) used for isolating the cellulose agreed well with the progressive formation of fibres in native grass leaves as illustrated in Fig. 9 and 16.

Whether the large increases in cellulose and lignin content of the cultivated grasses, especially brome, are really due to actual increases in lignin and cellulose content between the stages 3 and 4, or are only apparent increases caused by

(1) The polymer from the 10% solution was 100

solid on semi-solid pressed membranes, 10% 100

20.

(2) The process after may be more precise than that of process

when pressed owing to a relatively low initial content, 10%

2. According to Phillips (1954) a high initial content makes

plant tissues brittle but the exact concentration required

to reach this point is not stated.

The initial values obtained for speediness and 100

leaves, Stage 2, are worthy of special comment. They are almost

identical with those reported for Stage 1, Table 2. Unless the

initial lignin content of these grasses in 1949 was much lower

than that of 1949, little losses of soluble constituents have

occurred, otherwise the percentage of lignin would have

increased.

The results from the chromatographic method (10) were for

including the values agreed well with the progressive loss

of lignin in native grass leaves as illustrated in Fig.

and 10.

Whether the large increases in cellulose and lignin

content of the unfixed grasses, especially those, are really

due to actual increases in lignin and cellulose content or

are the stages 3 and 4, or are only apparent increases caused by

losses of the more soluble carbohydrates is not known since no chemical analyses were carried out to determine the latter constituents.

With respect to fat, no fatty acid determinations were carried out on the ether extracts so that whether the relatively high values for ether extract obtained from the native grasses, Stages 4 and 5, significantly affect energy value cannot be stated.

Since the results presented in the present studies for each grass are representative of one site and one year, only, further investigations must be carried out along the same lines, possibly with the addition of animal digestion trials before definite conclusions can be reached.

Recommendations for future investigation include the following:

- (1) That lignin, cellulose and ether extract be determined on samples of these four grasses, in the cured stage, from several sites for a number of years.
- (2) That fatty acid determinations be carried out on the ether extracts from the native grasses.
- (3) That soluble carbohydrate determinations be included with the above determinations.

Results of the more soluble compounds is not shown above
in chemical analysis were carried out in the same way as before
consistently.

With regard to the results of the determination of the
carried out on the same extracts so that neither the relatively
high values for these extracts obtained from the native material,
Stages 4 and 5, significantly affect the results. The results be
stated.

Since the results presented in the present section for
each group are representative of the site and the year, only
further investigations must be carried out when the results
possibly with the addition of special attention while before
definite conclusions can be reached.
Recommendations for future investigation include the

following:

- (1) That nitrogen, cellulose and other elements be determined on
samples of these four groups in the same way as before.
sites for a number of years.
- (2) That large scale determinations be carried out on the same
extracts from the native material.
- (3) That soluble carbohydrate determinations be included with
the above determinations.

SUMMARY AND CONCLUSIONS

Within the limits of this investigation there is reason to believe that curing is a combination of several factors, the most important of these being:

- (1) The position and the extent of the lignified tissues within the plant.
- (2) The presence or absence of reinforcing cellulose fibres which enable the plant to withstand weathering over a period of several months.
- (3) A relatively high fat content in the cured stage.

STUDY AND DISCUSSION

Within the limits of this investigation there is reason to believe that curing is a combination of several factors, the most important of these being:

- (1) The position and the extent of the lignified tissue within the plant.
- (2) The presence or absence of reinforcing cellular fibers which encase the plant to withstand weathering over a period of several months.
- (3) A relatively high fat content in the cured stage.

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