PALATABILITY AND DIGESTIBILITY OF GRASSES TREATED WITH GIBBERELLIC ACID

by

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INTRODUCTION

Perennial grasses are frequently inexpensive sources of feed for livestock. Along with other pasture plants they require less labor and are adapted to soils not suitable for other crops. These grasses are low in digestible energy content, relative to certain other feed sources. Some grasses, such as <u>Festuca arundinacea</u>, have the added disadvantage of being unpalatable to animals.

Gibberellic acid* is one of several forms of gibberellins occurring in some plants and microorganisms. When applied to many species of plants this class of compounds causes marked response in stem and leaf elongation. The rapid increase in plant cell elongation and/or division suggests a significant change in metabolism.

An increase in the digestibility and palatability of perennial grasses would make them more useful in supplying nutrients to livestock. When plant cell contents or cell wall structure are altered, the breakdown of cells by rumen microorganisms is affected. Plant growth regulators such as GA, which produce obvious morphological changes, would seem to cause some changes in cell make-up.

The research for this dissertation was initiated to study the effects of GA on the digestibility and palatability of two perennial grasses, orchardgrass and Kentucky 31 fescue.

^{*} Hereafter referred to as GA.

REVIEW OF LITERATURE

Influence of Gibberellic Acid on Plant Growth

The earliest recorded observation of the effect of gibberellins on plant growth is credited to Konishi, a semiliterate Japanese farmer, who dictated an agricultural book in 1809 (58). The effect was known as "bakanae" (foolish seedling) disease. It was caused by gibberellic acid produced by the fungus Gibberella fujikuori.

Gibberellins cause abnormal growth in a wide variety of plants.

The characteristic effect is an elongation of internodes. This has been reported in <u>Poa pratensis</u> (16, 22, 36, 38, 66), <u>Phaseolus vulgaris</u> (6, 8, 9, 25, 39, 40, 41, 60), <u>Zea mays</u> (42, 48, 49, 65), and many other species.

The increase in shoot growth by gibberellins is caused by increased cell elongation and/or multiplication. Stowe and Yamaki (58) reported that in rice an increase in cell elongation predominates over cell division. Kato (37) found this to be true in <u>Vigna sesquipedalis</u>. Greulach and Haesloop (25) concluded that the increase in height of pea plants (27.2 to 53.4 cm.) treated with GA was due to increased cell division rather than cell elongation. In a study of petiole growth in strawberry, cell multiplication and elongation contributed about equally to GA response. High light intensity increased GA stimulation (26). GA increased cell division and expansion in cotton embryo (19).

The mechanism by which GA stimulates plants is unknown. There is evidence that applied GA augments or substitutes for natural gibberellins.

Dwarf corn and peas are stimulated much more than normal strains (42). Phinney (48, 49) found that applications of 10, 15, and 20 micrograms of GA per plant caused single gene dwarf maize mutants to be as large as, and similar to, treated normal seedlings. When only one GA application was made, the dwarfs soon reverted to a very slow growth rate. A single gene double recessive dwarf of Lolium perenne was stimulated by GA to assume size and appearance similar to that of normal plants (15). Implications are that genetic dwarfs are deficient in natural GA. Lockhart (39) and Lockhart and Gottschall (4) studied the inhibition of dwarf pea stem growth by visible light compared to plants in darkness. GA overcame the inhibition by light, but caused little response in the dark. They concluded that light retarded synthesis or caused breakdown of GA in the plant. Brian and Hemming (7), using tissue cultures from pea stems, found no response to GA in the light unless an auxin was added. In another report (6) these authors reported on the effects of maleic hydrazide and GA on dwarf and normal peas. Maleic hydrazide inhibited growth in both plants. GA partially overcame the effects of maleic hydrazide on dwarf but not on tall peas. They concluded that the gene responsible for dwarfism in peas controls the production of a hormonelike substance similar to GA.

Influence of Gibberellic Acid on Composition of Plants

Less is known about the effects of GA on the chemical make-up of plants than about its effects on growth and morphology. Haber and Tolbert (27) working with peas, in short time metabolism studies, found GA did not affect photosynthesis and did not alter the pathways of newly

fixed C1402 in sugars, organic acids, and amino acid products. Ergle (21) applied GA to cotton seedlings; one mg. per plant doubled total sugars in the leaves and stems. GA also had the following effects: 1. lignin content was increased from 3.86 to 4.20% in the leaves and from 6.56 to 9.19% in the stems; 2. hemicellulose and cellulose contents were increased; and 3. nitrogen content was decreased by 1/4 in the leaves and 1/3 in the stems. Hayashi and Murakami (31) reported dry weight increases, but grain and root decreases when rice plants were grown in solutions containing 7 mg./l. of crude gibberellin. Hemicellulose, cellulose and lignin contents in the leaf sheaths were increased. Sucrose and starch were decreased by treatment. Wittwer et al. (66) found that GA applied to Kentucky bluegrass at the rate of 2 oz./A. reduced total sugar content from 8.65 to 6.09%. Brian et al. (5) reported slight increases in soluble carbohydrates of pea and wheat seedlings due to GA treatment. Dure and Jensen (19) found that GA applied to cotton embryos in vitro decreased the carbohydrate and nitrogen content per embryo and per cell.

The yield of a mixture of <u>Phalaris tuberosa</u> and <u>Trifolium subterraneum</u> was slightly increased by 3.69 oz./A. of GA (53). Protein content of the grass was decreased by GA; clover was unaffected in this respect. Treated grass and clover were both chlorotic. Finn and Nielsen (22) reported significant decreases in protein content of three grasses and three legumes when treated with 240 gm./A. of GA. Yields of tops were increased and roots decreased. Morgan and Mees (44) obtained increases in pasture yields of 10 to 25% at first harvest and

decreases of the same magnitude at the subsequent harvest by one application of GA. Nitrogen content of forage from the first harvest was decreased about 2%. Humphries and French (33, 34) found a decrease in nitrogen content of potato leaves after GA treatment. Applications of nitrate to the soil or urea to the leaves did not prevent the decrease in nitrogen. One application of 0.9 oz./A. of GA to a mixed sward in New Zealand increased yields at 6 weeks, but reduced regrowth (52). A parallel effect on nitrogen content was observed, although reduction at second harvest was insignificant.

Ortegon (45) sprayed alfalfa with GA concentrations of 25, 50, 100, and 200 ppm. The 25 and 50 ppm sprays increased yields and decreased protein content. Concentrations of 100 and 200 ppm decreased yields slightly and increased protein content. All differences in protein content were small.

Brian et al. (5) found a slight increase in nitrogen when pea and wheat seedlings were treated with GA. Arteta (2) applied up to 500 ppm concentrations of GA to sorghum plants grown with and without added nitrogen. When nitrogen was omitted, 500 ppm of GA increased protein from 4.74 to 7.59%. With nitrogen a similar but smaller effect was observed. Yield was not affected by GA. Nitrogen content of Kentucky bluegrass was not changed by GA (66).

Yermanos and Knowles (67) made foliar applications of GA to safflower, a plant grown for its seed oil. GA increased internode elongation, induced earliness, produced chlorosis, and reduced seed yield and oil content. Iodine number of the oil was unchanged. Howell

et al. (32) treated soybean seed with GA and obtained reduced stands and yields, with no effect on protein or oil content of the beans. Similar results on oil and protein content of corn grain were observed after spraying corn plants with GA solutions, Cherry et al. (14).

Caluya and Imlan (13) applied GA at rates from 0 to 100 ppm to

Hibiscus cannabinus L., a plant grown for its fiber content. Stem length
was increased markedly, but fiber content and the length and thickness
of fibers were not altered. Atal (3) treated hemp seedlings (Cannabis
sativa) with a 100 ppm GA spray on 3 successive days and then once weekly
for 10 weeks. Height of the plants was increased 247% by GA; stem
diameter increased 80%. Average fiber length increased from 0.4 to 3.75
cm., fiber thickness from 16.5 to 24.0, and fiber wall thickness from
4.5 to 7.5 as a result of GA treatment. At 6 and 10 weeks after
initiation of treatment fibers in treated plants were much more lignified.

Effect of Gibberellic Acid on Animals

Little data has been published on the effects of GA on animals. Sherman et al. (55) reported on a feeding trial in which GA was fed in pig starter rations at the rate of 0, 10, 100, and 1000 mg. per ton of feed. Rate of gain and feed conversion were unaffected. The same workers (28) also reported feeding GA to fattening lambs in amounts up to 1 gm. per ton of feed. In one of three trials GA increased rate of gain and feed efficiency slightly. Feeding chicks on a broiler-starter ration supplemented with 0.2, 2.0, or 20.0 gm. of potassium gibberellate per ton of feed produced no difference in growth rate, feed efficiency, or mortality (62).

Peck et al. (46) conducting toxicological research with GA gave rats and mice potassium gibberellate intravenously, orally by stomach tube and by feeding up to 8 weeks at a level of 5% of the diet. Non-specific signs of toxicity showed up when intravenous injections exceeded 4.2 gm. per kg. of body weight. Minimal signs of toxicity were observed with oral administration of 25 gm. per kg.

Cowan et al. (17) studied the effect of spraying alfalfa with GA solution 3 days prior to harvest on the performance of fattening lambs. Consumption, daily gain, and feed efficiency for lambs fed GA treated alfalfa were not different from those receiving untreated forage.

Factors Affecting Digestibility of Forages

The factors which affect the digestibility of forage plants have been extensively investigated. From the large amount of literature on this subject, conclusions can be drawn with respect to certain chemical fractions. An increase in crude fiber content of plants is almost invariably accompanied by a decrease in digestibility (23, 30, 43, 47, 56). The papers referred to here give correlations between crude fiber and dry matter digestibility of from -0.63 in Lespedeza cuneata (30) to -0.84 in timothy (47). Meyer and Lofgreen (43) obtained a correlation coefficient of -0.36 between crude fiber content of alfalfa and its IDN value. Protein content of forage was correlated with dry matter digestibility with coefficients varying from +0.71 with six grasses (56) to +0.966 with alfalfa (47). Lignin content was more closely correlated with dry matter digestibility, with coefficients ranging from -0.88 (56) to -0.97 (30).

Hawkins (30) found that nitrogen free extract (N.F.E.) and cellulose content of Lespedeza cuneata did not affect digestibility. Cellulose content of timothy and alfalfa was negatively correlated with energy and dry matter digestibility (47). Sullivan (59) studied digestibility of cellulose and dry matter in 36 grass hays and two legumes. Lignin content was highly correlated with digestion of true cellulose r = -0.92, natural cellulose r = -0.93, and dry matter r = -0.94. The percentage of alcohol-soluble substances was significantly correlated with dry matter digestion of 34 of the grass hays, r = +0.90. The inclusion of the other two grass hays, which had deteriorated on storage, and the two legumes, alfalfa and ladino clover, lowered this correlation coefficient to +0.65.

Factors Affecting the Palatability of Forages

Palatability of forages has been studied much less than digestibility. The factors controlling selectivity or intake of herbage are not understood. Grazing animals have been shown to consume herbage of higher nutritive value than the average for the total available herbage (4, 10, 11, 29). Measurements of nutritive value were digestibility, weight gain, and milk production. Proximate analysis data indicated selectivity was directly related to protein and ether extract and inversely to crude fiber content. N.F.E. content was not consistently related to selection. In one experiment (11) herbage selected by cattle during May and June was lower in N.F.E. than the total sward, but during August and September the cattle selected herbage higher in N.F.E.

Burton et al. (12) fertilized Coastal bermudagrass with nitrogen up to 1500 lb./A. Crude protein content increased as applied nitrogen increased. Palatability increased with applied N up to, but not beyond, 600 lb./A. Increases in palatability were paralleled by increases in moisture content and yield. Archibald et al. (1) compared seven grasses and found carotene and moisture content were closely related to palatability, followed by ether extract and ash content. Protein varied little and did not affect selection of herbage. Ivins (35) compared 12 species and found no relation between selection and chemical content.

Willard (64) tested palatability of the hay from 22 native grasses and 10 alfalfa cuttings. Selectivity in the grasses was highly related to sugar content. Alfalfa that browned in the stack lost sugar content, but retained palatability. Palatability was not consistently related to protein content. Plice (50) found that spraying several species of plants with sugar or molasses solutions improved palatability. Treatment with saccharine or sodium cyclohexyl sulfamate (artificial sweeteners) had the same effect. Analysis of wheat plants growing near droppings and refused by cattle showed that they contained almost twice as much protein but less sugar than plants unaffected by manure.

GENERAL PROCEDURE

Gibberellic acid was applied to grass in the field by spraying a 0.5% alcoholic solution of potassium gibberellate mixed with enough water to deliver the desired amount per acre. A manually operated sprayer (four-gallon capacity) with a six foot boom was used to apply the solution. One-half of the rate was applied in one direction and the remainder at right angles to the direction of the first application. In one experiment GA was applied in a granular form.

The lowest rate used was 12 gm./A., based on preliminary visual observations that this amount gave as much stimulation as higher rates. In later experiments the rates were increased in attempts to obtain more pronounced effects of GA on digestibility and palatability. The last application of GA in each experiment was made about two weeks prior to harvest.

Gibberellic acid treated and control areas were replicated in each experiment. This was accomplished by alternating GA treated and control areas. The grass harvested from the replicates was combined and mixed thoroughly before feeding. In statistical treatment of the results animals served as replications.

In all experiments, except one involving irrigation, grass was harvested with a garden-type tractor equipped with a five foot sickle bar mower. After raking the grass with hand rakes, it was dried at 175° F. in a forced air dryer. Grass from the irrigation experiment was mowed, raked and baled with farm tractor equipment. In this experiment

the grass was allowed to dry for a day in the field, then was baled loosely and dried at 175° F. in the forced air dryer.

In Experiments 1 and 2, the grass was fed in long form, but it was chopped (1-2" lengths) to facilitate feeding in subsequent experiments.

After chopping, the grass from each treatment was mixed and stored in cotton bags.

Sheep were used to test palatability and digestibility of the grass. The animals were one to two years old and ranged from 90 to 130 pounds in weight. In digestion trials, they were fed 800 gm. of air dry grass per day, except in one experiment, where this amount was not consumed; in this case, 750 gm. were fed. The grass was fed in two equal portions, one in early morning and one in late afternoon. Five gm. of salt were given at each feeding. The sheep had free access to water, except during feeding. Refused grass was collected after each feeding, then dried and weighed. All digestion trials employed seven day preliminary feeding periods, followed by seven days of total fecal collection. Feces were collected once daily and dried in a forced air oven at a maximum temperature of 60° C. for 24 hours. The total collection per sheep was composited and weighed after equilibration with the atmosphere for one week.

Grass was sampled by taking random hand grabs from each grass treatment at each feeding and compositing over the duration of the experiment. At the end of each experiment the composite sample was ground, mixed and subsampled for analysis. Feces were sampled by compositing the total collection, mixing, subsampling, and grinding in that order. The subsampling was done immediately after weighing. The samples were sealed

in glass bottles until analyzed. Feed and feces were ground to pass through a 60 mesh screen.

Chemical analyses of the grass and feces were made according to A.O.A.C. (1955) methods, except lignin, crude fiber, cellulose, and soluble carbohydrate determinations. Lignin was determined by the method described by Ellis et al (20); crude fiber by digesting an ether extracted sample with a mixture of acetic, nitric and trichloroacetic acids as described by Whitehouse et al (62); and cellulose by Crampton and Maynard's method (18). Soluble carbohydrate was measured by heating a 250 mg. sample in water at 100° C. for 45 min., filtering, and determining reducing sugars in the acid hydrolyzed filtrate by the method of Ting (60).

The palatability trials were conducted with the sheep used in digestion trials. Palatability in this study refers to the preference of the sheep for grass from one of two or three treatments. The grass was fed either in a fresh or fresh frozen state. When grass was fed fresh, it was given to the sheep immediately after harvesting. For other trials, it was frozen immediately after harvest and stored in the freezer until fed. Before feeding, the grass was thawed and chopped (1-2" lengths). Grass samples were taken at feeding time for moisture determinations. Intake was recorded as dry matter consumed.

Sheep were confined in individual pens and supplied with water.

Grass from treatments being compared was placed in the pen in separate containers. Number 2 laundry tubs were used as feed containers. Tubs were rotated at random in the pens, so selection was not confounded by

tub position. More grass from each treatment was offered than would be consumed. If all grass from one treatment was consumed at any feeding, that observation was disregarded in statistical analysis. The various trials differed in length and the number of animals used. In all cases, 4 to 5 days of preliminary feeding preceded measurement of palatability. The grass fed for palatability was essentially the same as that fed in the digestion work so chemical analyses were not made.

Analysis of variance was performed on the data from palatability and digestibility trials. Significance at either the 1 or 5% level of probability is indicated with each table of data. Where significance is not indicated, means were not different at the 5% level.

In the summer of 1959, two grass species (Experiments 1 and 2) were tested for palatability and digestibility following treatment with GA. Two species were used to determine the interaction, if any, between GA response and species.

Experiment 1 - The Effect of Gibberellic Acid on Palatability and

Digestibility of Summer Grown Kentucky 31 Fescue

PROCEDURE

Gibberellic acid absorbed into a granular clay material was applied to a fescue sod on May 29, 1959, at the rate of 12 gm./A. Fifty pounds of nitrogen was applied on June 25. The grass was mowed back to 2" on July 2. Due to dry weather and almost no growth by the grass, foliar application of GA was delayed until July 16. At this time 12 gm./A. of GA was applied to areas adjacent to the already treated ones, and similar areas were left untreated. There were two replications. On August 10, six additional gm./A. were applied to the sprayed area. The area with granular form received no additional GA. The GA treated grass was 10 to 12" high; untreated grass was 3 to 4" shorter.

A palatability trial was initiated on August 15. Six sheep were given a choice of fescue from the three treatments. This trial lasted eight days. Each day for each sheep was considered a replication, giving a total of 48 replications. The grass was cut fresh daily and fed at about 8:30 a.m. The feed containers were removed from the pens at about 7:00 p.m. and the uneaten grass dried, weighed and discarded.

On the day (August 15) that the palatability trial was begun, fescue was harvested from the same plots and dried in a forced air dryer for a digestion trial initiated on September 11. The experimental design was

a 3 x 3 latin square with three sheep. Each sheep was fed grass: 1. untreated, 2. treated with granular GA, and 3. treated with GA spray, in three separate runs. Sheep used in this experiment were confined in metabolism stalls.

RESULTS

Palatability:

When GA was applied to the sod in granular form there was a trend toward depression of grass consumption, Table 1. GA applied as a spray produced a much more pronounced and highly significant depression in intake. This difference in response to the two forms of GA is probably because sprayed grass received 1-1/2 times as much GA and the granular form was applied more than two months prior to the time the grass was harvested. In addition, one harvest of grass was taken from the granular treated plot on July 1. Total intake was low in this trial (621.6 gm./day). This is less than the amount of dry grass consumed in the digestion trial.

Table 1.--Consumption of Kentucky 31 Fescue as Influenced by Gibberellic Acid.

	Control	GA Granular 12 gm./A.	GA Spray 18 gm./A.
Intake (gm. dry matter per day)**	245.1 ^b	220.2 b	156.3 a

^{**} Means with different superscripts are different at the 1% level (C.V. - 36%).

Proximate composition of the grass fed is related to selective consumption, Table 2. Both protein content and palatability were decreased by GA spray. Crude fiber content was unchanged and N.F.E. was increased. Soluble carbohydrate appears to have been increased and ash content slightly depressed by GA. Moisture content of fresh grass was not affected by GA treatment. These composition data could not be subjected to statistical analysis. Treated grass was taller than the untreated and showed yellowing characteristic of GA treated plants.

Table 2.--The Effect of Gibberellic Acid on Composition of Kentucky 31
Fescue (percentage of dry matter, except moisture).

	Control	GA Granular 12 gm./A.	GA Spray 18 gm./A.
Protein	16.3	16.8	14.8
Ether extract	4.4	4.7	4.2
Crude fiber	28.7	27.1	28.7
Ash	7.7	7.6	6.8
Nitrogen free extract	42.9	43.8	45.4
Soluble carbohydrate 1	11.8	11.4	13.5
Moisture (at cutting)	78.6	78.8	79.0

Analysis of samples taken for moisture determination in palatability trial.

Digestibility:

Gibberellic acid tended to lower digestibility of fescue, Table 3.

All fractions of the grass tended to be lowered in digestibility by GA,
except ether extract. The granular form had less effect than the spray.

Only dry matter digestibility, however, was significantly depressed by
GA spray.

Table 3Digestibility	of	Kentucky	31	Fescue	as	Affected	by
Gibberellic A	cid	•					

	Digestion Coefficients %			
Fraction of Grass	Control	GA Granular 12 gm./A.	GA Spray 18 gm./A.	
Pry matter	66.4	65.7	63.6 **	
Protein	72.4	71.8	70.1	
Ether extract	54.9	57.9	57.0	
Crude fiber	69.7	68.2	66.8	
Nitrogen free extract	64.9	64.9	60.8	

^{**} Differs from the control at the 1% level.

The decrease in digestibility by GA spray coincides with a decrease in protein content. The level of protein in sprayed grass should not limit digestibility, however. The increase in N.F.E. content did not improve digestibility. There was a tendency toward a lowering of digestibility of crude fiber even though crude fiber content was not affected.

Crude fiber was more digestible than any fraction except protein.

N.F.E. was digested less than any fraction, except ether extract.

Experiment 2 - The Effect of Gibberellic Acid on the Palatability and

Digestibility of Summer Grown Orchardgrass

PROCEDURE

An established stand of orchardgrass was fertilized with 50 lb./A. of nitrogen on June 25, 1959. The grass was mowed to a 2" stubble on July 3. On July 16, two strips of sod were sprayed with 12 gm./A. of GA; alternate strips were left untreated. An additional application of 6 gm./A. was made on August 10.

A palatability trial was started on August 15, in which grass was fed to three sheep. The trial lasted eight days for a total of 24 replications. Orchardgrass was harvested when 10 to 12" high. Treatment with GA did not alter the height of grass.

A digestion trial was initiated on September 11 using grass harvested and dried on August 15; the procedure was similar to Experiment 1. A cross over design was used with four sheep. Two sheep received GA treated grass in run 1 and untreated in run 2. The other two sheep received treatments in the opposite sequence. In the second run only 700 gm. per day were fed due to a shortage of grass.

RESULTS

Palatability:

GA increased the palatability of orchardgrass as compared with untreated grass, Table 4. Proximate analysis indicate that GA did not affect the composition of orchardgrass, Table 5. The growing grass was not visibly affected by GA, except for slight yellowing. The differences in moisture and chemical make-up do not appear to account for the selection of GA treated grass.

Table 4 .-- Consumption of Orchardgrass as Affected by Gibberellic Acid.

	Control	GA, 18 gm./A.
Consumption (gm. dry matter per day)	451.2 g.	507.1 g. *

^{*} Differs from control at the 5% level (C.V. - 10.8%).

The variability in this trial was low, C.V. - 10.8%. Total consumption per day was considerably lower (958.3 gm.) than normal intake of sheep of this size (90 to 100 lb.).

Table 5.--The Effect of Gibberellic Acid on the Composition of Orchardgrass (percentage of dry matter, except moisture).

	Control	GA, 18 gm./A.
Protein	20.1	20.6
Ether extract	5.3	5.4
Crude fiber	27.5	27.7
Ash	7.5	7.0
Nitrogen free extract	39.0	39.8
Soluble carbohydrate 1	9.6	9.4
Moisture (at cutting)	78.3	77.4

Analysis of samples taken for moisture determination in palatability trial.

Digestibility:

Digestibility of orchardgrass was unaffected, except for N.F.E., which was slightly depressed by GA. With proximate composition unchanged by GA, a significant change in digestibility would have been unlikely. The significant depression of N.F.E. digestion may have been due to a slight increase in lignin content, much of which is included in the N.F.E. fraction in grasses (57). The same explanation may apply for the slight depression of crude fiber digestion (not significant).

	Digestion Coefficients %		
Fraction of Grass	Control	GA, 18 gm./A.	
Dry matter	65.4	64.2	
Protein	76.0	76.4	
Ether extract	45.0	45.0	
Crude fiber	70.6	68.3	

63.9

61.5 *

Table 6 .-- Digestibility of Orchardgrass as Affected by Gibberellic Acid.

Nitrogen free extract

Experiment 3 - The Effect of Gibberellic Acid on the Palatability and

Digestibility of Spring Grown Kentucky 31 Fescue

The spring growth of fescue is different physiologically from that produced in summer; stems and flower heads are present only in spring. The possibility of a different effect of GA on this type of growth led to an experiment using spring grown fescue. Because of the small differences due to GA treatment in 1959, the rate of application was increased in this experiment.

PROCEDURE

A fescue field, seeded in fall of 1959, was used. On April 22, 1960, 2,4-D was used to eliminate wild turnip from the sod. This herbicide was sprayed on after applying 500 lb./A. of 10-10-10 fertilizer.

The area was divided into four blocks; two were treated with GA; the remaining two were controls. GA was sprayed on the grass at the rate of 12 gm./A. on April 19 and on May 3. The grass used in the palatability and digestibility trials was harvested on May 12 while in

^{*} Differs from control at the 5% level.

the boot stage of growth. A portion of the harvested grass was immediately placed in a freezer in paper bags and later used for a palatability trial. Each bag contained enough grass for one feeding. Grass for digestibility determination was harvested on the same date, dried and stored for later feeding.

Palatability was tested in a trial started on July 10. Fresh frozen grass was chopped and offered to four sheep separately twice daily. The experiment consisted of six days for each of four sheep, giving 24 replications.

Digestibility of the spring grown grass was determined by feeding it to four sheep in a cross over trial, as in Experiment 2. The sheep were kept in one pen and fed from individual feed boxes. Their heads were yoked in the box during feeding and released afterward. Total fecal collection was made using a canvas bag and light harness. The trial was started when the sheep were accustomed to their feed boxes. Harnesses and bags were put on the sheep two days before the beginning of the first collection period to familiarize the animals with the equipment. The trial was started on June 12. Grass was chopped and mixed prior to this time.

RESULTS

Palatability:

Fescue treated with GA was lower in palatability than untreated grass. Sheep consumed 40% less GA treated grass than of the control, Table 7.

Table 7.--Consumption of Kentucky 31 Fescue as Affected by Gibberellic Acid.

	Control	GA, 24 gm./A.
Intake (gm. of dry matter per day)	501.1	304.7 **

^{**} Differs from control at the 1% level.

GA altered proximate composition slightly. Protein was decreased and N.F.E. increased. Both moisture content of the frozen samples and lignin in the dried grass were increased due to GA treatment. Soluble carbohydrate was about 6% higher in GA treated fescue. One fact, peculiar and unexplainable to this experiment, was the low ether extract content of the grass.

Table 8.--The Effect of Gibberellic Acid on the Composition of Kentucky 31 Fescue (percentage of dry matter, except moisture).

	Control	GA, 24 gm./A.
Protein	17.1	15.8
Ether extract	1.8	1.8
Crude fiber	24.6	25.0
Ash	8.5	8.0
Nitrogen free extract	48.0	49.3
Cellulose	24.8	25.0
Soluble carbohydrate	13.1	13. 9
Lignin	7.3	8.2
Moisture (at feeding)	69.4	71.7

Digestibility:

There was a trend toward a lowering of digestibility in this trial.

Dry matter, protein, crude fiber and cellulose were less digestible in the GA treated grass than in the control. These differences were not significant. N.F.E. digestion was unchanged and ether extract was made significantly more digestible by GA.

Table 9.--Digestibility of Kentucky 31 Fescue as Affected by Gibberellic Acid.

	Digestion Coefficients %		
Fraction of Grass	Control	GA	
Dry matter	63.3	62.2	
Protein	66.8	63.1	
Ether extract	30.1	35.5 *	
Crude fiber	66.9	65.2	
Nitrogen free extract	64.3	64.0	
Cellulose	68.9	67.7	

^{*} Differs from control at the 5% level.

Experiment 4 - The Effect of Gibberellic Acid and Irrigation on the

Palatability and Digestibility of Summer Grown Kentucky

31 Fescue

This experiment was conducted to compare the effects of GA on grass growing vigorously (irrigated) and grass growing at a much slower growth rate (unirrigated). A higher rate of GA was applied in this than previous experiments, in an attempt to increase the difference between GA and control grass.

PROCEDURE

The field of fescue used in Experiment 3 was divided into four sections; two sections were irrigated and the others were left dry.

Two divisions were made in each of the four sections; one division was sprayed with GA, the other left untreated. The entire area was mowed to a 2" stubble on June 13, 1960, and 50 lb./A. of nitrogen was applied, June 15. Applications of 24 gm./A. of GA were made on June 16 and July

23. Water was added to the irrigated plots by revolving sprinklers. When 50% of the water held by the soil at field capacity had been depleted (soil at 15% moisture), 1.75 to 2.00" of water were added; a total of 6.75" was applied between June 13 and August 3.

All grass was harvested for palatability and digestibility measurements on August 3. The grass, 51 days old, would have been harvested earlier, except for low yield of unirrigated grass. Grass yield from unirrigated plots was so low and grass so short that the farm rake used on other plots would not rake up the grass adequately. Hand rakes were used to gather the herbage. The unirrigated grass was wilted and appeared completely dormant. Although yields were not taken, the irrigated grass appeared to yield several-fold more than the unirrigated.

A palatability trial was started in the afternoon of the day that the grass was harvested. All of the feed for this trial was frozen and taken from the freezer as needed. The effects of GA and irrigation were tested separately. The effect of GA was tested by feeding treated and control grass from the irrigated plots. Four sheep were used and were offered the grass for five days. No measurement was made of the effect of GA on palatability of unirrigated grass, due to a shortage of this feed and because no significant effect due to GA was found on the irrigated grass. The effect of irrigation was tested by feeding untreated grass from irrigated and dry plots to four sheep, for three days. The trial was of short duration because difference in consumption between irrigated and non-irrigated grass was large and consistent.

Digestibility of irrigated and unirrigated grass, each with and without GA, was tested in a 4 x 4 latin square. Each of four sheep received grass from one of the four treatments in four separate runs. The procedure was similar to that in Experiment 3, with harnesses and bags being used to collect feces.

RESULTS

Palatability:

GA did not significantly alter palatability of the irrigated grass, Table 10. There was a tendency to select GA treated grass, but the difference was not significant, possibly because of the large amount of random variability (coefficient of variability = 51.5%). GA treated grass had received a total of 48 gm./A. Moisture content was very little affected by GA treatment, Table 12. GA tended to decrease protein and ether extract and increase crude fiber and cellulose content in the irrigated grass. These trends were not observed for non-irrigated grass.

Table 10.--Consumption of Irrigated Kentucky 31 Fescue as Affected by Gibberellic Acid.

	Control	GA, 48 gm./A.
Intake (gm. of dry matter per day) 1	573.6	630.8

¹ Means not statistically different, (C.V. - 51.5%).

Selection for irrigated grass was very noticeable in the palatability trial in which the effect of irrigation was measured (Table 11). This may be due to the large difference in moisture content. Protein, ether

extract and lignin were decreased by irrigation; crude fiber and cellulose were slightly increased (control columns, Table 12).

Table 11. -- Consumption of Kentucky 31 Fescue as Affected by Irrigation.

	Non-Irrigated	Irrigated
Intake (gm. dry matter per day)	349.2 **	704.6

^{**} Differs from irrigated at the 1% level, (C.V. - 51.7%).

Table 12.--The Effect of Gibberellic Acid and Irrigation on the Composition of Kentucky 31 Fescue (percentage of dry matter, except moisture).

	Non-I	rrigated	Irrigated		
	Control	GA 48 gm./A.	Control	GA 48 gm./A.	
Protein	14.1	14.1	13.7	12.1	
Ether extract	5.1	5.1	4.3	3.5	
Crude fiber	24.8	24.6	26.1	27.8	
Ash	8.0	7.4	7.7	7.7	
Nitrogen free extract	47.9	48.7	48.2	48.9	
Cellulose	25.8	25.6	26.6	28.2	
Lignin	10.3	8.6	7.8	7.6	
Soluble carbohydrate	15.2	16.8	16.7	15.9	
Moisture (at feeding)	52.2	50.7	73.7	74.6	

¹ Applies only to the palatability trial.

Digestibility:

Irrigation caused a small and non-significant increase in digestibility as compared with non-irrigated grass, Table 13. Irrigation tended to increase digestibility of dry matter, ether extract, crude fiber, nitrogen free extract and cellulose, though none of the increases were large enough to be significant. Only protein and ether extract showed a trend toward increased digestibility by GA. The digestibility of protein in irrigated grass and ether extract in irrigated and unirrigated grass was depressed by GA.

Table 13.--Digestibility of Kentucky 31 Fescue as Affected by Gibberellic Acid and Irrigation.

	Digestion Coefficients %					
	Non-I	rrigated	Irrigated			
Fraction	Control	GA 48 gm./A.	Control	GA 48 gm./A.		
Dry matter	61.0	62.9	63.6	64.7		
Protein	64.2	64.8	63.5	59.8		
Ether extract 1	39.2 ab	34.6 b	43.1 ^a	38.6 ab		
Crude fiber	65.3	66.3	67.9	70.4		
Nitrogen free extract	62.0	64.8	64.8	65.8		
Cellulose	66.5	68.7	67.6	70.1		

¹ Coefficients not having same superscripts differ at the 5% level.

While irrigation tended to increase digestibility of crude fiber, nitrogen free extract, and cellulose, both crude fiber and cellulose contents were increased by irrigation. An increase in crude fiber content is usually associated with a decrease in its digestibility. The apparent discrepancy is possibly explained by the decrease in lignin content caused by irrigation.

Experiment 5 - The Effect of Gibberellic Acid and Age on the Digestibility of Fall Grown Kentucky 31 Fescue

This experiment was conducted to study GA effect in the fall season when growth rate is slow, but when forage quality is frequently high.

An additional factor, age of grass, was studied, as well as the interaction between GA and age of grass.

PROCEDURE

Grass from the area used in Experiment 4 was harvested and discarded on September 2, 1960. Fertilizer (10-20-10) was then applied at the rate of 500 lb./A. On September 6, strips of sod not treated with GA in Experiment 4 were sprayed with 24 gm./A. of GA. Control strips in the same sections (not receiving GA in Experiment 4) were left untreated. Two acre-inches of water were applied to the sod on September 9. Portions of the treated and control areas were harvested on September 19 and October 3, and 24 to obtain grass 2, 4, and 6 weeks of age.

Additional GA (24 gm./A.) was applied to the unharvested grass on September 20 and October 5. The 4 and 6 week old grass received totals of 48 and 72 gm./A. of GA, respectively.

Palatability was not studied. Digestibility was determined by feeding six sheep in a replicated 3 x 3 latin square design. Each of two squares employed three sheep and three ages of grass. Sheep in one square received GA treated grass; the other group were fed untreated grass. The difference between squares included the GA effect. Sheep were assigned to the two groups at random. Feeding and fecal collection procedures were the same as for Experiments 3 and 4. Only 750 gm. of grass were fed to each sheep, because 800 gm. were not consumed in preliminary feedings.

RESULTS

GA decreased protein, ether extract, and lignin contents, but increased crude fiber, N.F.E., and cellulose, Table 14. Ash, as in previous experiments, was decreased by GA. As grass aged from 2 to 6 weeks protein, crude fiber, cellulose, lignin, and ash contents decreased and N.F.E. and soluble carbohydrate increased. There was very little interaction between the effects of GA and age of grass.

Table 14.--The Effect of Gibberellic Acid and Age on the Composition of Kentucky 31 Fescue (percentage of dry matter).

			Age of Gr	ass (Weeks)		
	2		4		6	
	Control	GA 24 gm./A.	Control	GA 48 gm./A.	Control	GA 72 gm./A.
Protein	18.7	15.9	16.9	12.4	13.4	10.7
Ether extract	3.4	3.2	4.0	3.2	3.8	3.2
Crude fiber	27.8	29.6	25.5	27.5	24.2	25.6
Ash	9.5	9.2	9.4	8.2	8.5	7.7
Nitrogen free						
extract	40.5	42.2	44.2	48.6	50.1	52.7
Cellulose	31.1	34.1	28.6	31.3	26.9	29.3
Lignin Soluble	9.6	8.7	8.1	7.5	6.5	5.2
carbohydrate	9.2	9.4	14.3	13.7	21.4	20.9

Age of the grass affected digestibility of every organic component, Table 15. The digestibilities of dry matter, ether extract, and N.F.E. increased with age. With increased age of the grass, protein, crude fiber, and cellulose became less digestible. The increase in dry matter digestibility with age is due to the increases in digestibility of N.F.E. and, to a lesser extent, ether extract. The increase in digestibility of ether extract may be due to more non-chromogen material in this

fraction of 4 and 6 week old grass. Increases in soluble carbohydrate and decreases in lignin content are undoubtedly the reasons for the higher digestibility of the N.F.E. in older grass.

Reasons for the decline in digestibility of crude fiber and cellulose with age are not readily apparent. It seems that these fractions would increase in digestibility as lignin content drops, since lignin is closely associated with them in plant tissue. The explanation may be that the increases in carbohydrate content as grass became older furnished the sheep with a more digestible energy source, thus reducing the dependence on energy from fiber. Digestion of the fibrous fractions did not drop except in the 6 week old grass, when N.F.E. made up 50% of the dry weight of the plants. Lignin could not have made up much of the N.F.E. fraction in this case.

The less digestible protein in the older grass may be explained by the difference in nitrogen nutrition of the grass. Fifty pounds of nitrogen per acre, applied when the experiment started, was probably absorbed quickly, raising the protein content of the grass. It has been shown that increased nitrogen fertilization increases the protein and its digestibility in orchardgrass (51). Uptake of nitrogen at later stages may have been limited by a dwindling nitrogen supply and perhaps by lower temperatures.

Table 15.--Digestibility of Kentucky 31 Fescue as Affected by Gibberellic Acid and Age.

Age of Gr	ass (Wks.			gestion Coefficients 4		6	
Fraction	Control	GA 24 gm./A.	Control	GA 48 gm./A.	Control	GA 72 gm./A	
Dry matter	68.6	67.8	71.6	69.8	71.2	70.0	
Protein	69.9	69.8	72.0	66.5	58.9	63.9	
Ether extract	43.7	47.7	49.8	51.0	53.3	57.4	
Crude fiber Nitrogen free	75.8	75.0	76.7	74.6	74.8	70.4	
extract	71.0	65.1	72.5	70.5	75.7	72.6	
Cellulose	78.6	76.4	80.5	76.3	76.3	73.3	

Differences due to age, significant at the 5% level (all fractions). Differences due to GA, significant at the 5% level (all fractions, except protein).

Treatment of the grass with GA caused trends in this experiment similar to those in previous ones: digestibility of all fractions, except ether extract and protein was depressed; ether extract digestion was favored; and protein was unaffected. GA slightly decreased ether extract at each age of grass, and may have changed its composition. The color change caused by GA in all of the experiments indicates a change in plant pigment composition and/or content. A change in composition of ether extract is indicated by the differences in its digestibility.

Depression of crude fiber, N.F.E., and cellulose digestion by GA does not appear to be related to lignification. GA treatment reduced lignin content. Barring a difference in mode of lignin deposition in cell walls, differences in digestion of these fractions caused by GA are likely related to changed content or kind of carbohydrates involved.

In this experiment crude fiber and cellulose were more digestible than N.F.E. in grass of 2 and 4 weeks of age. In 6 week old grass, there

was no difference. This indicates a chemical change within one or more of these fractions with age; an increase in soluble carbohydrates in the N.F.E. is most probable.

DISCUSSION

Chemical Composition:

Preliminary to the discussion of GA effects on palatability and digestibility, the changes in chemical composition will be reviewed.

The chemical change commonly found in GA studies, depression of protein content, occurred in four out of five experiments. The exception was orchardgrass sprayed with GA at 18 gm./A. The degree of depression in protein content varied from 7.3% in the spring of 1960 to an average of 18.2% in the fall of that year. Reductions in protein may have been a dilution effect, increased yield with nitrogen uptake unchanged.

Ortegon (37) found that GA rates which increased alfalfa growth also reduced protein. High rates of GA decreased yield and increased protein content.

Ether extract was decreased by GA in two out of five experiments and unchanged in three. The change in color of grasses in all experiments after applying GA indicated there were changes in kind or amount of pigments. Ether extract may have been changed in composition as indicated by usual increases in digestibility. This fraction makes up such a small portion of the grass and is so indigestible that it contributes little to dry matter digestibility or nutritive value.

Crude fiber content was not affected by GA except in the summer and fall experiments of 1960, where the increases were low, 6.5%. Cellulose content was affected in the same way as crude fiber in these two experiments; and was not affected in the other experiment in which it was

measured (spring 1960). The increases in cellulose caused by GA (6.2 and 9.2%) agree with the observations of early Japanese workers (57) and Ergle's work with cotton seedlings (21).

Increases in lignification, due to GA treatment, have been reported (27, 57). Lignin was increased 11.8% by GA in spring grown fescue. In the summer of 1960, GA treatment caused lignin reductions of 16.5 and 3.2% in the unirrigated and irrigated grass, respectively. Lignin content of 2, 4, and 6 week old grass was depressed by GA 8.9, 8.3, and 20.2%, respectively, in the fall of that year. Crude fiber and lignin contents are usually closely correlated. In two experiments GA did not affect the two components in the same way. The reason for this may be that crude fiber contained most of the cellulose in the plants and little of the lignin. Stallcup (67) found that crude fiber from Kentucky 31 fescue contained 88.6% of the total cellulose and only 31.0% of the lignin. He used the original crude fiber procedure as opposed to the acid-mixture digestion (62) used in this work.

Since nitrogen free extract contains most of the lignin of grasses, contents of these two fractions might be expected to shift in the same direction when altered by GA. N.F.E., however, was consistently higher in the GA treated than in the control grass. The increases were small, ranging from 1.5 to 6.4%. These increases may have been due to an increase in hemicellulose, most of which appears in the N.F.E. Ergle (21) found GA to double hemicellulose in cotton leaves and increase it 23.3% in the stems and petioles. The small increases in percentage of N.F.E. may be significant since this fraction makes up 40 to 50% of the dry matter. In

two experiments where GA increased N.F.E. about 6%, it reduced digestibility of dry matter about one-half as much. Ash content of the grass was decreased by GA in every case. The decreases were variable, however, from almost none (0.2%) in the summer of 1960 to 10.9% in the summer of 1959. This, as with protein, may be due to increased yield with GA (dilution effect).

Kentucky 31 fescue was very similar in composition in all of the experiments. Ether extract varied more among experiments than any other component. Except for ether extract, the composition of grass differed more due to age in the fall 1960 experiment than between experiments.

It was postulated that irrigation would produce marked changes in the composition of the fescue. While growth rate and yield were greatly increased by irrigation, change in composition was slight. Deferring of harvest until the unirrigated grass yielded enough for the trials may have confounded the effect of irrigation, as grass was 51 days old when cut. Irrigated grass was much higher (74%) in moisture content than unirrigated grass (51%) when cut. There was no dew on the morning of harvest and the unirrigated grass was wilted. On mornings when there was considerable dew, the grass appeared normal, but wilted again by midmorning.

Unirrigated grass was short, brittle when dry, and appeared to be of very poor quality. Proximate analysis showed that it was slightly higher in protein and lower in crude fiber than irrigated grass. Lignin and ether extract were decreased by irrigation. The small difference in digestibility between irrigated and unirrigated grass indicates that quality was only slightly affected.

Decreases in crude fiber, cellulose and lignin with age of fall grown fescue are contrary to most literature reports for spring grown grass. As fescue aged from 2 to 6 weeks, cellulose content dropped about 14%, but N.F.E. increased by about 24%. The changes in percentages of crude fiber, lignin, cellulose, and N.F.E. may be explained by the increase in soluble carbohydrate content with age. Increase in N.F.E. from 2 to 4 and 6 week old grass is matched almost exactly by increases in soluble carbohydrate. Crude fiber, cellulose and lignin percentages may have dropped due to decreased synthesis and dilution as soluble carbohydrate accumulated. These changes indicate that the metabolism in fall grown grass differs from grasses grown in spring and summer.

Palatability:

Palatability, as discussed here, refers to selection among grasses fed simultaneously and gives no indication of intake differences that might occur if grasses were fed separately. The latter kind of data might have more practical value, but would probably be affected much less by GA.

Intake of grass in all of the palatability trials, except in the summer of 1960, was low for sheep of this size (approximately 100 pounds). In two out of three trials fescue was made less acceptable to sheep by applying GA. Intake of treated grass was about 38% less than for the control. In the third trial selection was in favor of GA treated fescue, but the difference was not significant. It is possible that the age of the grass (51 days) reduced the influence of GA in this trial.

Limited data with orchardgrass indicate an interaction between grass species and the effect of GA on palatability. GA treated orchardgrass was preferred; consumption was about 12% higher for treated than control grass. Irrigated grass was much more palatable than unirrigated; 66.8% of daily intake was from the irrigated grass.

Data obtained do not explain palatability differences. The protein in fescue was consistently depressed by GA, yet in the summer of 1960 the higher protein, untreated grass, was not selected. The increased consumption of orchardgrass caused by GA was not related to a protein increase. Comparative palatabilities of irrigated and unirrigated fescue show higher consumption of irrigated grass even though it was lower in protein.

Moisture may have been the dominant factor in the selection of irrigated grass since irrigation increased moisture content 45% compared to unirrigated grass. Moisture content probably does not determine total daily intakes, however. In Experiment 1, only 621 gm. of dry matter as fresh fescue was consumed in the palatability trial, but 800 gm. of air dry (approx. 10% moisture) grass was eaten in the digestion trial.

Moisture content of grass was affected little by GA and may not have been a factor in the selection between treated and control grass. Soluble carbohydrate content does not appear to be related to palatability, since GA had only a slight effect on this component and the grass selected was lowest in soluble carbohydrate. Other work indicates a positive correlation between total sugar content and palatability (63).

GA may alter palatability through a change in some ether soluble flavor component. Also, some physical property of the grass, such as tensile or compression strength, roughness of the surface or blade edges may be changed by GA.

Digestibility:

The stimulation of stem and leaf elongation by GA is due to an increase in cell division and/or elongation rate. It seems that an increase in either might affect the susceptibility of the cells to microbial attack in the rumen, since the plant cell metabolism is probably changed in rapid division or elongation.

There was a trend for GA treated grass to be lower in digestibility than untreated grass in four out of five experiments. The differences in dry matter digestibility were significant in two experiments. GA treated grass was 2.0 to 4.2% lower in dry matter digestibility than untreated grass. In one experiment GA treatment tended to increase digestibility, but the differences were not significant.

Digestibility of ether extract did not follow the pattern of dry matter digestion, and was not correlated with ether extract concentration in plants. In one experiment where GA treated grass was more digestible than untreated, digestibility of ether extract was lowered by GA. In other experiments, GA effects ranged from zero to a 17.9% increase in ether extract digestion. Since GA had no consistent effect on ether extract content, differences were probably due to changes in composition of the ether extract. A decrease in pigment or chromogen synthesis in favor of more digestible fatty substances would help explain the effect of GA on digestibility of the ether extract and yellowing of the grass.

Digestibility of protein was not significantly changed by GA. The average digestion percentage was lowered by GA in four out of eight comparisons, unchanged in three and increased in one. Protein content was decreased by GA in six out of eight comparisons.

There was a tendency for crude fiber digestibility to be lower in GA treated than control grass. The difference ranged from 1.0 to 5.9% depression in four experiments, but was significant in only one. GA increased crude fiber content less than 2%, except in one experiment where the increase was 6.8%. This was the experiment where depression in crude fiber digestibility was significant.

Crude fiber digestion in one experiment was slightly higher (3.7%) for the GA treated grass, though crude fiber content was also higher (6.5%). Lignin content was decreased by GA in this experiment, which may explain the increase in crude fiber digestion.

In the fall of 1960, lignin content was decreased and crude fiber increased by GA. Crude fiber was less digestible in GA treated than control grass. The compositional data do not explain this difference. It is possible that GA alters the polymerization or deposition of lignin in the cell walls. Differences in components other than lignin may have influenced crude fiber digestion.

Cellulose digestibility was about the same as crude fiber both in magnitude and reaction to GA. This is probably due to the fact that the crude fiber in these experiments was mostly cellulose. In spring and summer of 1960 the contents of the two fractions were almost identical.

N.F.E. digestibility tended to be decreased by GA in all of the experiments, except in the summer of 1960. Decreases ranged from 0.5 to 8.3% and were significant in two experiments. The reasons for GA reducing digestibility of N.F.E. are not clear. The effect may be due to a change in some component of the N.F.E. which was not measured, possibly hemicellulose, since this component was greatly affected in cotton (21). A decrease in lignin in the GA treated fall grown grass did not make N.F.E. more digestible. GA did not affect soluble carbohydrate content in this experiment. Although the protein level was fairly high in the grass, its reduction by GA may have affected digestibility of N.F.E. as well as some of the other fractions.

Irrigation increased digestibility of fescue slightly. The probable cause was a decrease in lignin content due to irrigation. Analysis showed slightly less protein and more crude fiber and cellulose in the irrigated grass. The unirrigated grass appeared to be of poor quality, but it contained 14.1% protein and was low in fiber (24.7%).

Dry matter digestibility of fescue grown in the fall of 1960 increased from 68.2 to 70.6% as grass aged from 2 to 6 weeks. This increase was significant and the 4 week old grass had a digestibility value between the two. Protein, crude fiber and cellulose became less digestible with age. The latter fractions decreased in digestibility in spite of a 35% decrease in lignin content from 2 to 6 weeks. Slight decreases in lignin usually coincide with significant increases in digestibility of the fibrous portions of forages (59). The opposite relationship in this experiment is probably explained by the high soluble carbohydrate content (20%) of

the older grass. This carbohydrate probably furnished the rumen microorganism population with a readily available energy source, making it less dependent on cellulose or crude fiber.

N.F.E. and ether extract digestibility increased with age, 9.0 and 21.0%, respectively. The increase in digestion of N.F.E. was undoubtedly caused by the increase in soluble carbohydrate and decrease in percent lignin in this fraction. The increased digestibility of ether extract with age may be due to a change in the make-up of this fraction, since its concentration did not change greatly. Conditions of advancing season in the fall may favor formation of digestible fats over indigestible plant pigments, both of which are contained in this fraction.

SUMMARY AND CONCLUSIONS

Experiments were conducted to study the effect of gibberellic acid on palatability and digestibility of two grasses, orchardgrass and Kentucky 31 fescue. The GA was applied as a spray, except one granular application, at rates of 18 to 72 gm./A. Sheep were used to test the grass for palatability and digestibility. In addition to proximate analysis of grass, data were obtained on soluble carbohydrate, cellulose and lignin contents.

Chemical composition of orchardgrass was not affected by GA treatment. The most consistent effect of GA on the chemical composition of fescue was a depression of protein content. Ash content was decreased slightly, but consistently. GA increased crude fiber in two experiments; did not affect it in three. Nitrogen free extract was increased slightly in three of the five tests. Cellulose was increased by GA in the summer and fall of 1960, but not in the spring. Lignin was increased in the spring of that year and decreased in the summer and fall. Ether extract and soluble carbohydrate contents were not consistently affected by GA. In general, chemical changes by GA were less than 5%.

Orchardgrass palatability was improved by GA. In two out of three trials with Kentucky 31 fescue, consumption of GA treated grass was about 38% less than for the control. Irrigation increased palatability, probably due to a large difference in moisture content of the grass. No chemical differences observed were consistently related to selection of grass by the sheep.

Digestibility of dry matter in grass tended to be decreased by GA in four out of five experiments. These depressions were less than 5% and in only two cases were they significant. Digestibility of protein, crude fiber, nitrogen free extract and cellulose tended to be decreased and ether extract was made more digestible by GA. Due to the small changes in both digestibility and chemical composition caused by GA no clear cut explanations could be given for the reduction of digestibility. The effect seems related to a slight protein reduction and in some cases to an increase in crude fiber and cellulose.

Although a number of factors which may affect GA response (time after application, growth rate of grass, light intensity, temperature, etc.) have not been studied, it seems unlikely that this growth regulator will exert much effect on the factors controlling digestibility of grass under field conditions. The effect on palatability is more pronounced, but not consistent.

The digestibility of fall grown fescue increased with age. This increase probably resulted from accumulation of soluble carbohydrates and decreases in crude fiber, cellulose, and lignin contents in the grass.

LITERATURE CITED

- Archibald, J. G., E. Bennett and W. S. Ritchie. The composition and palatability of some common grasses. J. Agr. Res. 66: 341. 1943.
- Arteta, de la H. E. Algunos efectos de la aspersion foliar de acido giberelico en sorgo (Sorghum vulgare Pers.). Acta Agron.
 10: 1. 1960.
- 3. Atal, C. K. Effect of gibberellin on the fibers of hemp. Econ.
 Bot. 15: 133. 1959.
- 4. Blaser, R. E., R. C. Hammes, Jr., H. T. Bryant, W. A. Hardison, J. P. Fontenot, and R. W. Engel. The effect of selective grazing on animal output. Proc. 8th Intern. Grassl. Cong. 601. 1960.
- 5. Brian, R. W., G. W. Elson, H. G. Hemming, and M. Radley. The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus <u>Gibberella fujikori</u>. J. Sci. Fd. Agr. 5: 602. 1954.
- 6. Brian, R. W. and H. G. Hemming. The effect of maleic hydrazide on the growth response of plants to gibberellic acid. Ann. App. Biol. 45: 489. 1957.
- 7. Brian, R. W. and H. G. Hemming. Complimentary action of gibberellic acid and auxins in pea internode extension. Ann. Bot. N. S. 22: 1. 1958.

- 8. Brian, R. W., H. G. Hemming and D. Lowe. Effect of gibberellic acid on rate of extension and maturation of pea internodes.

 Ann. Bot. N. S. 22: 539. 1958.
- 9. Brian, R. W., H. G. Hemming and D. Lowe. Effect of gibberellic acid on shoot growth of Cupid sweet peas. Physiol. Plant. 12: 15. 1959.
- 10. Bryant, H. T., R. E. Blaser, R. C. Hammes, Jr., and W. A. Hardison.

 Method for increased milk production with rotational grazing.

 J. Dairy Sci. 44: 1733. 1961.
- 11. Bryant, H. T., R. E. Blaser, R. C. Hammes, Jr., and W. A. Hardison.

 Comparison of continuous and rotational grazing of three

 forage mixtures by dairy cows. J. Dairy Sci. 44: 1742. 1961.
- 12. Burton, G. W., B. L. Southwell and J. C. Johnson, Jr. The palatability of Coastal bermudagrass (Cynodon dactylon (L.) (Pers.)) as influenced by nitrogen level and age. Agron. J. 48: 360.
 1956.
- 13. Caluya, M. F. and J. S. Imlan. Effect of gibberellic acid on the fiber of kenaf. Phillipine Agriculturist 43: 369. 1960.
- 14. Cherry, J., H. A. Lund and E. B. Earley. Effect of gibberellic acid on growth and yield of corn. Agron. J. 52: 167. 1960.
- 15. Cooper, J. P. The effect of gibberellic acid on a genetic dwarf of Lolium perenne. New Phytol. 57: 235. 1958.
- 16. Corns, W. G. Effects of foliage treatments with gibberellic on forage yield of alfalfa, Kentucky bluegrass and winter wheat.
 Can. J. Pl. Sci. 38: 314. 1958.

- 17. Cowan, R. L., E. Keck, Jr., R. C. Miller, T. L. Merrit, and J. H. Ziegler. Effect of pelleting and gibberellic acid on the nutritive value of alfalfa by fattening lambs. (Abstract)

 J. Ani. Sci. 21: 137. 1962.
- 18. Crampton, E. W. and L. A. Maynard. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutr. 15: 383. 1938.
- 19. Dure, L. S. and W. A. Jensen. The influence of gibberellic acid and indoleacetic acid on cotton embryos cultured in vitro. Pl. Physiol. Supplement 32: XXXI. 1957.
- 20. Ellis, G. H., G. Matrone and L. A. Maynard. A 72% H₂SO₄ method for the determination of lignin and its use in animal nutrition studies. J. Ani. Sci. 5: 285. 1946.
- 21. Ergle, D. R. Compositional factors associated with the growth responses of young cotton plants to gibberellic acid. Pl. Physiol. 33: 344. 1958.
- 22. Finn, B. J. and K. F. Nielsen. Effects of gibberellin on forage yields of six grass and legume species. Can. J. Pl. Sci. 39: 175. 1959.
- 23. Forbes, R. M. and W. P. Garrigus. Some relationships between chemical composition, nutritive value, and intake of forages grazed by steers and weathers. J. Ani. Sci. 9: 354. 1950.
- 24. Gray, R. A. Alteration of leaf size and shape and other changes caused by gibberellins in plants. Amer. J. Bot. 44: 674. 1957.

- 25. Greulach, V. A. and J. D. Haesloop. The influence of gibberellic acid on cell division and cell elongation in <u>Phaseolus vulgaris</u>.
 Amer. J. Bot. 45: 566. 1958.
- 26. Guttridge, C. G. and P. A. Thompson. Effect of gibberellic acid on length and number of epidermal cells in petioles of strawberry. Nature 133: 197. 1959.
- 27. Haber, A. H. and N. E. Tolbert. Photosynthesis in gibberellintreated leaves. Pl. Physiol. 32: 152. 1957.
- 28. Hale, W. H., W. C. Sherman, W. M. Reynolds, and H. G. Luther.

 Evaluation of gibberellic acid in lamb rations. (Abstract)

 J. Ani. Sci. 17: 1172. 1958.
- 29. Hardison, W. A., J. T. Reid, C. M. Martin, and P. G. Woolfolk.

 Degree of herbage selection by grazing cattle. J. Dairy Sci.

 37: 89. 1954.
- 30. Hawkins, G. E. Relationships between chemical composition and some nutritive qualities of <u>Lespedeza sericea</u> hays. J. Ani. Sci. 18: 763. 1959.
- 31. Hayashi, T. and Y. Murakami. Physiological action of gibberellin.

 Nogyo Gijitsu Kenkyuyo Hokoku. Ser. D. 7: 159. 1958. (from

 Chem. Abstracts 52: 20431. 1958.)
- 32. Howell, R. W., C. J. Worgel, C. A. Brim, E. E. Hartwig, J. W.

 Lambert, J. R. Thompson, B. R. Stefansson, J. K. Park, W. E.

 Seigler, and B. K. Webb. Response of soybeans to seed-treatment with gibberellin under simulated commercial conditions.

 Agron. J. 52: 144. 1960.

- 33. Humphries, E. C. and S. A. W. French. The effect of gibberellic acid on leaf area and dry matter production in Majestic potato.

 Ann. App. Biol. 48: 177. 1960.
- 34. Humphries, E. C. and S. A. W. French. Effect of nitrogen supply on the response of Majestic potato to gibberellic acid. Ann. App. Biol. 49: 331. 1961.
- 35. Ivins, J. D. The relative palatability of herbage plants. J. Brit. Grassl. Soc. 7: 43. 1952.
- 36. Juska, F. V. The effect of gibberellic acid on Kentucky bluegrass root production. Agron. J. 51: 184. 1959.
- 37. Kato, Y. Responses of plant cells to gibberellin. Bot. Gaz. 117: 16. 1955.
- 38. Leben, C., E. F. Alder and A. Chichuck. Influence of gibberellic acid on the growth of Kentucky bluegrass. Agron. J. 51: 116.
- 39. Lockhart, J. A. Studies on the mechanism of stem growth inhibition by visible radiation. Pl. Physiol. 34: 457. 1959.
- 40. Lockhart, J. A. and V. Gottschall. Growth response of Alaska

 pea seedling to visible radiation and gibberellic acid. Pl.

 Physiol. 34: 460. 1959.
- 41. Marth, C., W. V. Audia and J. W. Mitchell. Effects of gibberellic acid on growth and development of plants of various genera and species. Bot. Gaz. 118: 106. 1956.
- 42. McCune, D. C. and A. W. Galston. Inverse effects of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn. Pl. Physiol. 34: 416. 1959.

- 43. Meyer, J. H. and G. P. Lofgreen. The estimation of the total digestible nutrients in alfalfa from its lignin and crude fiber content. J. Ani. Sci. 15: 543. 1956.
- 44. Morgan, D. G. and G. C. Mees. Gibberellic acid effect and the growth of crop plants. Nature 178: 1356. 1956.
- 45. Ortegon, E. Tres respuestas de la alfalfa (Medicago sativa L.)

 a la applicación de acido giberelico. Acta Agron. 10: 57.

 1960.
- 46. Peck, H. M., S. E. McKinney, A. Tytell, and B. B. Byham. Toxicological evaluation of gibberellic acid. Science 126: 1064.

 1957.
- 47. Phillips, T. G. and M. E. Laughlin. Composition and digestible energy of hays fed to cattle. J. Agr. Res. 78: 389. 1949.
- 48. Phinney, B. O. Biochemical mutants in maize-dwarfism and its reversal with gibberellins. Pl. Physiol. Supplement 31: XX. 1956.
- 49. Phinney, B. O. Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Nat. Acad. Sci. 42: 185.

 1956.
- 50. Plice, M. J. Sugar versus the intuitive choice of foods by livestock. J. Range Mgt. 5: 69. 1952.
- 51. Poulton, B. R., G. J. MacDonald, and G. W. VanderNoot. Effect of nitrogen fertilization on the nutritive value of orchardgrass hay. J. Ani. Sci. 16: 462. 1957.

- 52. Scott, R. S. Effect of gibberellic acid and nitrogen on winter growth of pasture. New Zea. J. Agr. Res. 2: 1203. 1959.
- 53. Scurfield, G. and E. F. Biddiscombe. Effects of gibberellic acid on winter pasture production. Nature 183: 1196. 1959.
- 54. Scurfield, G. and C. W. E. Moore. Effect of gibberellic acid on species of Eucalyptus. Nature 181: 1276. 1958.
- 55. Sherman, W. C., N. D. Magruder, W. M. Reynolds, and H. G. Luther.

 Nutritional studies with gibberellin in swine rations.

 (Abstract) J. Ani. Sci. 17: 1161. 1958.
- 56. Sosulski, F. W. and J. K. Patterson. Correlations between digestibility and chemical constituents of selected grass varieties. Agron. J. 53: 145. 1961.
- 57. Stallcup, O. T. Composition of crude fiber in certain roughages.

 J. Dairy Sci. 41: 963. 1958.
- 58. Stowe, B. B. and T. Yamaki. The history and physiological action of the gibberellins. Ann. Rev. Pl. Physiol. 8: 181. 1957.
- 59. Sullivan, J. T. Cellulose and lignin in forage grasses and their digestion coefficients. J. Ani. Sci. 14: 710. 1955.
- 60. Thurber, G. A., J. R. Douglas, and A. W. Watson. Inhibitory effect of gibberellins on nodulization in dwarf beans, <u>Phaseolus</u> vulgaris. Nature 181: 1082. 1958.
- 61. Ting, S. V. Rapid colorimetric method for simultaneous determination of total reducing sugars and fructose in citrus juices. J. Agr. Fd. Chem. 4: 263. 1956.
- 62. Warden, W. K. and P. J. Schaible. Effect of gibberellic acid in broiler-starter rations. Poultry Sci. 37: 490. 1958.

- 63. Whitehouse, D., A. Zarow, and H. Shay. Rapid method for determining "crude fiber" in distillers' dried grain. J. Assoc. Off. Agr.

 Chem. 28: 147. 1945.
- 64. Willard, H. S. Factors affecting the palatability of hay. Wyo. Agr. Exp. Sta. Bul. 199. 1953.
- 65. Wittwer, S. H. and M. J. Bukovac. Gibberellin effects on temperature and photoperiodic requirements for flowering of some plants.

 Science 126: 30. 1957.
- 66. Wittwer, S. H., M. J. Bukovac and B. H. Grigsby. Gibberellin and higher plants: VI. Effects on the composition of Kentucky bluegrass (Poa pratensis) grown under field conditions in early spring. Mich. Agr. Exp. Sta. Quart. Bul. 40: 203. 1957.
- 67. Yermanos, D. M. and P. F. Knowles. Effects of gibberellic acid treatment on safflower. Agron. J. 52: 596. 1960.

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ABSTRACT

Palatability and Digestibility of Grasses Treated with Gibberellic Acid

by

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Experiments were conducted to study the effect of gibberellic acid on palatability and digestibility of two grasses, orchardgrass and Kentucky 31 fescue. The GA was applied as a spray, except one granular application, at rates of 18 to 72 gm./A. Sheep were used to test the grass for palatability and digestibility. In addition to proximate analysis of grass, data were obtained on soluble carbohydrate, cellulose and lignin contents.

Chemical composition of orchardgrass was not affected by GA treatment. The most consistent effect of GA on the chemical composition of fescue was a depression of protein content. Ash content was decreased slightly, but consistently. GA increased crude fiber in two experiments; did not affect it in three. Nitrogen free extract was increased slightly in three of the five tests. Cellulose was increased by GA in the summer and fall of 1960, but not in the spring. Lignin was increased in the spring of that year and decreased in the summer and fall. Ether extract and soluble carbohydrate contents were not consistently affected by GA. In general, chemical changes by GA were less than 5%.

Orchardgrass palatability was improved by GA. In two out of three trials with Kentucky 31 fescue, consumption of GA treated grass was about

38% less than for the control. Irrigation increased palatability, probably due to a large difference in moisture content of the grass. No chemical differences observed were consistently related to selection of grass by the sheep.

Digestibility of dry matter in grass tended to be decreased by GA in four out of five experiments. These depressions were less than 5% and in only two cases were they significant. Digestibility of protein, crude fiber, nitrogen free extract and cellulose tended to be decreased and ether extract was made more digestible by GA. Due to the small changes in both digestibility and chemical composition caused by GA no clear cut explanations could be given for the reduction of digestibility. The effect seems related to a slight protein reduction and in some cases to an increase in crude fiber and cellulose.

Although a number of factors which may affect GA response (time after application, growth rate of grass, light intensity, temperature, etc.) have not been studied, it seems unlikely that this growth regulator will exert much effect on the factors controlling digestibility of grass under field conditions. The effect on palatability is more pronounced, but not consistent.

The digestibility of fall grown fescue increased with age. This increase probably resulted from accumulation of soluble carbohydrates and decreases in crude fiber, cellulose, and lignin contents in the grass.