EVALUATION OF FRONTIER, RIVAL AND VENTURE REED CANARYGRASS AS PASTURE FORAGE

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by

Gerald William Duynisveld

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GERALD WILLIAM DUYNISVELD

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ABSTRACT

Two studies were conducted to evaluate Rival, Venture and Frontier reed canarygrass (RCG) and Champ timothy as pasture forage using growing lambs and to determine if alkaloid type and concentration accounted for any differences identified. In order to determine the effects of gramine and hordenine, a quantification system was developed using gas-liquid chromatography.

In 1987, fresh frozen initial growth and regrowth of each cultivar were fed to forty lambs for determination of dry matter (DM) intake and then to sixteen ram lambs for determination of apparent digestibility of DM, acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP). Content of CP, ADF, NDF, calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), sulfur (S), zinc (Zn), copper (Cu), gramine and hordenine were determined for forage of each cultivar. No differences ($P>0.05$) were observed between timothy and RCG cultivars or between cultivars of RCG for dry matter intake of either initial growth or regrowth. There were differences ($P<0.05$) among cultivars for DM, CP, ADF and NDF digestibility in both initial and regrowth. Apparent DM and fiber digestibility of Rival was higher ($P<0.05$) than Frontier and Venture for initial and regrowth. Champ timothy generally had higher ($P<0.05$) DM and fiber digestibilities when ADF levels were similiar to RCG cultivars. Gramine and hordenine concentrations of RCG cultivars were low in both initial growth and regrowth, and seem to have no effect on intake or digestibility.

In 1988, weight gains of ten lambs grazing in each of two paddocks for each of the cultivars were monitored from May 25 to July 5, period I and from July 30 to August 30, period II. Forage CP, ADF, NDF, gramine

and hordenine contents were determined weekly and used to create nonlinear regression equations for each component in each paddock. Contents of Ca, P, Mg, S, Cu and Zn were determined at three dates in each period. Lambs grazing Champ were removed from test due to the hot dry conditions which caused inadequate forage production in these paddocks. There were no differences ($P>0.05$) in nutrient and alkaloid concentration among cultivars of RCG in period I. In period II, there were differences ($P<0.05$) among cultivars for gramine and fiber concentrations. Differences were observed ($P=0.07$) in average daily gains (ADG) of lambs grazing the three cultivars: means (± SE) were 119, 146 and 161 ± 8 g for Frontier, Rival and Venture, respectively. An interaction ($P=0.06$) was detected between cultivar and period, reflecting changes in forage alkaloid concentration under drought conditions. Seven lambs from each cultivar were placed on a finishing ration, after removal from the grazing trial, to determine if any residual effects of the cultivars were present. No differences ($P>0.05$) in ADG were detected among lambs from different cultivars when placed on a finishing ration suggesting that the alkaloids had no residual effects.

In samples collected during 1988, no differences ($P>0.05$) were detected in hordenine concentration due site location, stage of growth or cultivar. Gramine contents were higher ($P<0.05$) in regrowth than initial growth. Rival had higher ($P<0.05$) gramine concentrations than Venture with Castor, Palaton and Vantage being intermediate. No differences ($P>0.05$) in gramine content were detected between two off site locations.

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TABLE OF CONTENTS

LISTS OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Page

ABREVIATIONS

ADF	acid detergent fiber
ADG	average daily gain
BPE	bovine pulmonary emphysema
BW	body weight
BSFTA	bis(trimethylsilyl)-trifluoracetamide
°C	degree celcius
Ca	calcium
cm	centimeter
CP	crude protein
Cu	copper
CWC	cell wall content
df	degrees of freedom
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DMT	N,N-dimethyltryptamine
g	grams
GLC	gas-liquid chromatography
ha	hectare
HCL	hydrochloric acid
hr	hour
ID	inner diameter
IU	international unit
IVDDM	invitro digestible dry matter
K	potassium
kg	kilogram

ul	microliter
l	liter
m	meter
Mg	magnesium
mg	milligram
min	minute
mm	millimeter
um	micrometer
MTHC	2-methyl-1,2,3,4-tetrahydro-β-carboline
n	number of observations
N	nitrogen
Na	sodium
NDF	neutral detergent fiber
NMT	N-methyltryptamine
P	phosphorus
RCG	reed canarygrass
rpm	revolutions per minute
RSD	residual standard deviation
S	sulfur
SD	standard deviation
SE	standard error
TC	tryptamine and β-carboline
wt	weight
Zn	zinc
5MeO-DMT	5methoxy-N,N-dimethyltryptamine
5MeO-NMT	5methoxy-N-methyltryptamine
6MeO-DTHC	2,9-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline

6MeO-THC   2-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline

INTRODUCTION

Reed canarygrass (RCG), Phalaris arundinaceae, is a cool season, perennial grass which has shown much potential as a forage. It is adapted to a wide range of growing conditions and is very persistent in low lying areas. RCG is tolerant of flooding and yet shows good drought tolerance (Marten 1973). In low lying areas, it is high yielding and has a good distribution of growth through spring and summer because of its continual state of tiller production (Smith et al. 1973). RCG is suited to either a pasture or hay situation. Dry matter production of RCG is very responsive to nitrogen fertilization and recovery of applied nitrogen is higher than most grasses (Marten et al. 1979).

Wide acceptance of RCG has not occured due to problems associated with forage palatability and poor performance of animals consuming the grass (Marten 1973). These responses are due to the alkaloids which have been detected in RCG. Alkaloids are nitrogen containing compounds with a complex ring structure. These compounds are produced in plants and show significant pharmalogical activity. Nine alkaloids have been detected in RCG: hordenine, gramine, four tryptamines and three β-carbolines. Hordenine is the only non-indole alkaloid detected in RCG.

Palatability of RCG has been correlated ($r=-0.094$) with total indole alkaloid concentration (Simons and Marten 1971). Weight gains of animals consuming RCG have been found to be influenced by alkaloid type and concentration (Martens et al. 1976, 1981). Digestibility and intake of RCG were also observed to be influenced by alkaloid type and concentration when high concentrations of synthetic alkaloids were added to animal feed (Arnold et al. 1980).

The presence of the tryptamine and β-carboline alkaloids is controlled genetically. Genotypes containing only gramine and hordenine have been isolated (Woods and Clark 1971a,b). Recent cultivars have been developed to be free of tryptamines and β-carbolines and have reduced levels of gramine. Problems associated with RCG, such as diarrhea and reduced animal gains, should be minimized with the use of these cultivars.

Two studies were conducted to evaluate three cultivars of RCG and one cultivar of timothy as pasture forage. Venture and Rival are two RCG cultivars which are free of the tryptamine and β-carboline alkaloids and were registered in 1985 and 1987, respectively. Frontier, a RCG cultivar which contains tryptamine and β-carboline alkaloids, was licensed in 1959. Champ, a cultivar of timothy licensed in 1967, was included as an alkaloid free pasture grass.

Intake and apparent digestibility of DM, crude protein and fiber components were evaluated in the first animal study. The forage was evaluated as fresh frozen initial or reproductive growth and the sebsequent or regrowth ie. first and second cut. The second study evaluated the productivity of animals grazing the four cultivars for two periods of six weeks. An additional study developed a technique to quantify gramine, hordenine and 5-methoxy-N,N-dimethyl tryptamine using gas-liquid chromatography. Performances of the animals consuming the different cultivars were interpreted taking into consideration alkaloid type, alkaloid concentration and nutrient profiles.

LITERATURE REVIEW

Description of Reed Canarygrass

Reed canarygrass (RCG), *Phalaris arundinacea* L., is a long lived, cool season, perennial grass. Culms can reach 200 cm in length, appear coarse and can bear up to 9 leaves, each 2 cm wide and up to 30 cm long. Culms of RCG need vernalization to produce seed and therefore regrowth remains vegetative. The inflorescence is a semi-dense panicle which opens for pollination and then closes. Seeds mature downward from the top of the panicle and shatter immediately after ripening. RCG grows as dense clumps and can spread by short rhizomes to form a dense sod.

Agronomic Characteristics of Reed Canarygrass

Adaptation

One of the main attributes of RCG is its ability to withstand ponding for up to two months. It is tolerant of water logged soils and is commonly found along margins of streams and ponds in North America, Europe, and Asia. Reed canarygrass produces well on upland soils with ample moisture and also exhibits drought tolerance. It can be grown in a wide range of soil pH (4.9-8.3) but does not tolerate salinity. However, seedlings are slow to establish and are not tolerant of flooding (Bittman et al. 1980).

Dry Matter Yield

Dry matter production of perennial forages is influenced by a number of factors which include stage of growth at cutting, number of cuts per year, local environment and soil fertility. Most of the data presented on RCG productivity is from growth under clipping either as hay or pasture simulation. There is very little data on RCG yield under pasture conditions.

Dry matter yields of first cut RCG increased ($P<0.05$) to the seed set stage over two years in British Columbia. However, cutting of RCG at earlier stages of initial growth increased ($P<0.05$) the yield of the second cut. This change in distribution of yield during the season did not affect ($P>0.05$) total seasonal dry matter yield when first cut ranged from heading to seed set (Bonin and Tomlin 1968). Decker et al. (1967) found no consistent relationship between RCG total seasonal dry matter yield and growth stage at cutting when determined at four locations over three years in the Northeastern USA. Under "favorable conditions", they found RCG could produce 13.5 tonne ha^{-1}. Stands of RCG were observed to have good production in mid to late summer but poor production in the fall (Schmid et al. 1979; Fairney 1985). The benefit of RCG as a forage crop is its flexibility in distribution of yield because cutting initial growth early allows a good yield of regrowth for late summer and does not affect total seasonal dry matter yield.

Comparison of RCG to other grass species for dry matter production is difficult to interpret due to factors such as stage at cutting, number of cuts per season and locations at which these species are evaluated. Generally, most evaluations of yield differences between various species are conducted such that all species are cut on the same day and not necessarily at the same stage of growth. A study by Marten and Hovin (1980) compared four grasses cut 2, 3 or 4 times per growing season. In the first year, two of the four grass species were in boot stage while the other two were vegetative on the day of cutting of initial growth. All four were vegetative in the following year. In the last year, two were vegetative and the other two were in the boot stage. These differences in stage of growth at cutting may cause bias if comparisons are made between species for first cut because dry matter yield of first cut RCG increases until seed set stage (Bonin and Tomlin 1968).

Marten and Hovin (1980) compared dry matter yield of RCG, tall fescue, orchardgrass, and smooth bromegrass cut 2, 3 or 4 times per year at one location. The only yield difference ($P<0.05$) observed between species in all three years was that RCG outyielded smooth bromegrass. Mean yields were 10.5 and 8.9 tonne ha^{-1}, respectively. Schmid et al. (1979) found no consistent relationship for dry matter yield of six grass species at 10 locations over three years. These researchers suggested that if statistical tests had been applied, a significant interaction between location and species would have occurred. In this study, tall fescue and orchardgrass were generally among the highest yielding species, kentucky bluegrass yielding lowest, with smooth bromegrass, RCG and timothy being interdispersed. In British Columbia, orchardgrass outyielded ($P<0.05$) perennial ryegrass, hybrid ryegrass and RCG under a four cut system during the two years of the test (Fairney 1985). Mean yields for the species were 11394, 10148, 10164 and 10049 kg ha^{-1}, respectively. As the number of locations where comparisons were made increased, differences between environments were a greater influence on dry matter yield than differences between species. Therefore, comparisons between species for yield should be made at the location where the species are intended to be grown and under management practices to be experienced.

Nitrogen generally is the most limiting nutrient to grass growth. Niehaus (1971) found dry matter yield of RCG responded positively to application rates as high as 600 kg N ha^{-1} but the largest yield increases kg^{-1} fertilizer were at lower application rates. This author also found 75 kg N ha^{-1} to be insufficient for proper plant growth, based upon the light green color of the RCG plants. Under Manitoba conditions, Dean and Clark (1972) found 224 kg N ha^{-1} applied at the beginning of the growing season under a two cut system to be optimum for maximum nitrogen use efficiency. If three cuts per year were taken, 336 to 448 kg N ha^{-1} would be considered optimum.

Kline and Broersma (1983) compared seasonal yields of RCG, meadow foxtail and timothy in British Columbia and found average yields over four years increased ($P<0.05$) with the application of nitrogen fertilizer. The yields of RCG and meadow foxtail were still increasing with the application of 360 kg N ha^{-1}. Dry matter yield of timothy reached its maximum at 180 kg N ha^{-1}. Unfertilized timothy yielded twice as much dry matter as unfertilized RCG. They also found there was no yield increase ($P>0.05$) with split fertilizer application as compared to single spring application. For RCG to be productive, substantial amounts of nitrogen should be applied.

<u>Persistence</u>

Persistence or longevity of the stand is important to a producer because of the difficulty in establishing a stand of grass and the need to spread possible losses in income from the year of establishment over a longer period of time. Reducing the length of time between cuts tends to increase quality of the harvested forage, however this can reduce plant reserves needed for regrowth leading to a possible reduction in stand persistence.

Marten and Hovin (1980) observed RCG to persist well at 2, 3 or 4 cuttings per year over three years. Tall fescue stands were damaged when cut twice yearly possibly due to shading of stem bases which would interfere with regrowth. Orchardgrass persistence was reduced as the cutting interval was lengthened because of its sensitivity to cutting below the collar and elongation of the collar usually occurred when cut twice yearly. Bromegrass persistence was increased when cut twice yearly.

Jung et al. (1974) compared increasing the cutting frequency to 3, 5 or 8 times per year. Bromegrass and RCG persistence was favored with clipping 3 times per year with added nitrogen. Kentucky bluegrass, tall fescue, orchardgrass and timothy persistence improved with more frequent clipping especially with added nitrogen. Clipping 2, 3 or 4 times per year can be used as an indication of persistence under a hay or rotational grazing situation whereas 5 to 8 cuts per season would be an indication of persistence under continuous or short rotation grazing. Interpretation of the number of cuts per season is also dependant on the local growing season. In Western Canada, there are usually two cuts with the possibility of a third cut of fall growth whereas in Eastern Canada and the Eastern USA, 3 to 4 cuts can be expected.

Comparisons of persistence when cut 3 or 4 times per year have been used to evaluate the potential grass species for mixtures with legumes. Cutting dates are set to coincide with the harvesting dates for alfalfa. However, the persistence of grasses in monoculture may differ when the grasses are grown with a legume. Persistence of RCG grown in monoculture is good when cut 3 or 4 times per year (Jung et al. 1974; Marten and Hovin 1980) but when grown with alfalfa and cut 3 times per year, Jones et al. (1988) found the percent alfalfa rose from 56.7 to 99.5 by the eighth cut. However, when grown with birdsfoot trefoil, the trefoil remained between 67.3 to 80.7 % of stand after eight cuts. These authors suggested that the faster rate of recovery of alfalfa caused shading and increased the interspecific competition against RCG. Smith et al. (1973) found RCG, tall fescue and orchardgrass to have better persistence than bromegrass and timothy when grown with alfalfa and cut three times per year. They suggested the reduction of bromegrass and timothy stands was due to the fact that basal tillers were usually arrested until the elongating shoots were near anthesis. Thus growth had to be reinitated from the crown whereas RCG, tall fescue and orchardgrass appeared to be in a continual state of tiller production. The persistence of RCG would be of benefit to producers because stand density only becomes reduced if the cutting or grazing interval is very short. Persistence when grown with a legume depends on the vigour of legume regrowth.

Crude Protein Content

Protein in the diet of the ruminant is necessary for the replacement and production of body tissues. Determination of crude protein (CP) or N*6.25 includes the amount of protein nitrogen and non-protein nitrogen, both of which can be utilized by the ruminant depending on the degree of lignification of each. The CP content of first cut RCG decreases with maturity (Decker et al. 1967). Bonin and Tomlin (1968) observed first cut CP yield of RCG did not differ ($P>0.05$) when cut from shot blade stage to anthesis, but before or after this period CP yield of first cut decreased ($P<0.05$). Total CP yield for the season did not differ ($P>0.05$) if initial growth was cut from the vegetative stage to heading, but delaying first cut past the heading stage caused a decrease ($P<0.05$) in CP yield. Therefore, cutting of RCG was recommended at the boot to heading stage to maximize dry matter yield with the least reduction in CP content. Higher levels of applied nitrogen caused CP yields to reach maximum levels at earlier growth stages and CP yields were increased ($P<0.05$). Niehaus (1971) found CP levels to be affected ($P<0.05$) by cultivar of RCG only once out of three years. Nitrogen fertilization increased both dry matter yield and CP content with CP yield increasing at an even faster rate.

Levels of CP in RCG were higher than bromegrass, timothy, orchardgrass, kentucky bluegrass and tall fescue at two locations over two years (Schmid et al. 1979). Values of CP were 21.0, 19.6, 18.3, 17.3 and 17.6 %, respectively, but no mention was made of stage of maturity at cutting. Marten et al. (1979) found RCG to be superior to other species in extraction of nitrogen from applied sewage effluent because it produced the highest CP yield even though it did not always have the highest dry matter yield.

Marten and Hovin (1980) suggested CP content of RCG declined more rapidly due to maturation than for orchardgrass or tall fescue. This was suggested from the observation of the decline in CP content as the interval between cuttings increased. Jordan and Marten (1975) determined pasture forage of RCG, bromegrass and orchardgrass to have CP contents of 30.0, 28.2 and 27.6 %, respectively. Donker et al. (1976) observed five week regrowth of alfalfa and RCG to be of similar CP content. Values of CP content of pasture forage are generally higher than that found in hay if the pasture is grazed such that plants remain in a vegetative state. Initial growth CP yield is a compromise between CP content and yield because the CP levels are decreasing while dry matter yield is increasing as the plant matures. Generally, RCG is regarded as a grass species with high CP content when fertilized with nitrogen and utilized before heading.

Fiber Content and Digestibility

Much of the carbohydrate in a grass forage is found in fiber or cell walls. The availability or digestibility of the carbohydrates depends on its form and degree of lignification. The use of the detergent system to estimate the various portions of fiber in feedstuffs has been in use since the mid 1960's. Cell wall content (CWC) was estimated by neutral detergent fiber and includes the fiber components of hemicellulose, fiber bound protein, cellulose, lignin and lignified nitrogen. It has been observed that CWC was negatively correlated (-.76) with voluntary dry matter intake (Van Soest et al. 1978).

Donker et al. (1976) determined the CWC to be much higher in RCG than in alfalfa due to a higher amount of hemicellulose in RCG. Tall fescue has been observed to have higher ($P<0.05$) levels of CWC than RCG and smooth bromegrass two out of three years (Marten and Hovin 1980). Acid detergent fiber (ADF) estimates cellulose, lignin and lignified nitrogen and has been observed to be negatively correlated (-.75) with dry matter digestibility (Van Soest et al. 1978). Donker et al. (1976) found higher levels of ADF in RCG than in alfalfa, although no stage of maturity at cutting was mentioned. Lignin, determined as acid detergent lignin, was much higher in alfalfa than RCG (Donker et al. 1976). Lignin will decrease the digestibility of any component that it is complexed with. Marten et al. (1981) found differences ($P<0.05$) between three cultivars of RCG sampled from pastures for CWC but not for lignin or ADF.

In vitro digestible dry matter (IVDDM) is a method used to estimate the dry matter digestibility (DMD) of a feedstuff by ruminants; the correlation for the two being 0.80 (Van Soest et al. 1978). The yield of RCG IVDDM for first cut was higher ($P<0.05$) when cut at heading to anthesis than at other growth stages. Nitrogen application increased ($P<0.05$) IVDDM yield. The seasonal yield of IVDDM was higher ($P<0.05$) if the first cut was taken at the boot to heading stage than at other stages (Bonin and Tomlin 1968). Thus, it was recommended that harvesting occur at boot to heading.

Niehaus (1971) found small differences in IVDDM between three cultivars of RCG although no statistical analysis could be performed. However, the rankings were consistent over three years: Frontier > common > Ioreed. Mean invitro DMD of the three cultivars was found to decrease from first to second to third cut with values of 71.4, 66.0 and 57.6 %, respectively. Marten et al. (1981) observed no difference ($P>0.05$) between three cultivars of RCG for IVDDM. The cultivars were Rise, Vantage and Mn-76 sampled from pastures. Values of IVDDM of RCG in pasture have been determined as high as 80 to 85 % (Jordan and Marten 1975; Marten et al. 1976).

In comparison with other grasses, RCG was found to have a faster rate of reduction for IVDDM content as cutting frequency was reduced i.e. if RCG digestibility was similar to another grass species under a four cut system, it would be lower than the other species under a three or two cut system (Marten and Hovin 1980). In British Columbia, orchardgrass and perennial ryegrass produced more IVDDM than RCG with hybrid ryegrass being intermediate (Fairney 1985). There were differences ($P<0.05$) in IVDDM content with the ranking of perennial ryegrass, hybrid ryegrass, RCG and orchardgrass. Values were 66.9, 64.3, 63.2 and 59.9 %, respectively.

Ingalls et al. (1965) found no differences ($P>0.05$) between DMD of alfalfa, birdsfoot trefoil, smooth bromegrass and RCG over two cuts and two years when fed to sheep as dried hay. The values for the four species were 60.4, 61.4, 62.5 and 61.7 %, respectively. First cut for all four species was more digestible ($P<0.05$) than second cut. Donker et al. (1976) observed no difference ($P>0.05$) in DMD of alfalfa and RCG when fed as fresh cut or dried hay to sheep. Crude protein digestibility was higher ($P<0.01$) in alfalfa even though crude protein levels were similar. Crude fiber digestibility was higher ($P<0.05$) in RCG due to the greater lignification of crude fiber in alfalfa.

When compared to alfalfa, RCG has a higher CWC due to its higher hemicellulose and ADF content, however lignin content is much lower. Therefore, intake is expected to be lower than alfalfa but digestibility of fiber components should be higher whereas DMD may or may not be lower.

Mineral Content

Mineral content of plant material is influenced by soil availability of the mineral. Ranges in the literature for calcium, phosphorus, potassium, magnesium, copper, zinc and molybdenum in RCG on a dry matter basis are 0.2-0.4 %, 0.2-0.5 %,2-4 %, 0.2-0.4 %, 5-30 mg kg^{-1}, 20-50 mg kg^{-1}, and 0.5-2.0 mg kg^{-1} (Barnes and Mott 1970; Dean and Clark 1972; Jordan and Marten 1975; Marten et al. 1981). The upper range of copper concentration maybe toxic to sheep (NRC 1985). The minerals listed individually meet requirement for sheep except for calcium, which for growing lambs should be twice as high as phosphorus (NRC 1985).

Barnes and Mott (1970) compared alfalfa and RCG for mineral content and found alfalfa to be higher ($P<0.05$) in calcium, copper, and boron. Potassium, manganese and zinc were higher ($P<0.05$) in RCG than alfalfa whereas phosphorus, magnesium, iron, and copper were not different ($P>0.05$) between the two species.

Alkaloids of Reed Canarygrass

Alkaloids Detected in Reed Canarygrass

The nine alkaloids found in RCG can be classified by their ring structure: phenethylamine found in hordenine or indole amine found in gramine, the four tryptamines and the three β-carbolines (Marum et al. 1979). The first alkaloids to be detected in RCG were 5-methoxy-N-methyl-tryptamine (5MeO-NMT) and hordenine by Wilkinson (1958). Gramine and 2,9-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-DTHC) were identified by Culvenor et al. (1964) and Audette et al. (1970), respectively. Several research groups (Barnes et al. 1971; Williams et al. 1971; Woods and Clark 1971b) identified 5-methoxy-N,N-dimethyltryptamine (5MeO-DMT) and N,N-dimethyltryptamine (DMT) in RCG samples. N-methyl-tryptamine (NMT), 2-methyl-6-methoxy-tetrahydro-β-carboline (6-MeO-THC) and 2-methyl-1,2,3,4-tetrahydro-β-carboline (MTHC) were identified by Woods and Clark (1971b), Marten (1973) and Gander et al. (1976), respectively.

Woods and Clark (1971b) found RCG plants which were free of tryptamines and suggested that the presence of the tryptamines was controlled by a single dominant gene. Marum et al. (1979) classified seven of the eight indole alkaloids found in RCG to three phenotypic groups based on chemical structure and biosynthesis: the T group contains NMT, DMT, MTHC and gramine; the MeO group includes 5MeO-NMT, 5MeO-DMT, 6MeO-THC and gramine; and the G group contains only gramine. The 6MeO-DTHC was not assigned to any of the three groups due to lack of information but its structure is related to the MeO group. Hordenine was not included in this model because of its phenolic structure.

Marum et al. (1979) suggested a two gene model for the expression of the phenotypic groups. The dominant allele M expresses the MeO phenotype and the dominant T allele at the second locus expresses the T phenotype with the G phenotype being expressed when both alleles are recessive (MeO=M___, T=mmT_, G=mmtt). They also determined that M was epistatic and linked to T. Therefore, selection for tryptamine and β-carboline free genotypes is simplified because of the double recessive.

Coulman et al. (1976) detected a wide variation in gramine concentration between plants of the G phenotype. These authors found a high correlation between years and between first growth ($r=0.82$) and regrowth ($r=0.72$) for plant gramine concentration. Woods et al. (1979) observed broad sense heritability estimates of both gramine and hordenine for first growth and regrowth forage. Correlations between gramine and hordenine for concentration and seasonal variation were statistically significant ($P<0.05$) but only slightly positive. Therefore, there is potential for the development of cultivars free of tryptamines and β-carbolines with low levels of either gramine or hordenine but not necessarily both.

<u>Function</u>

The exact function of the alkaloids is highly debated. They are possibly the waste products of metabolism which accumulate because plants lack an efficient excretory system. They may also be a defence mechanism against grazers, insects and disease. Gramine inhibited the germination of powdery mildew (<u>Erysphe graminis</u> f.sp. <u>hordei</u>) in barley (Wippich and Wink 1984). Gramine also has been shown to reduce susceptibility of barley seedlings to aphids (<u>Rhodalsiphum padi</u>) (Zuniga and Courcuera 1986). Alkaloids can also serve as intermediates in metabolic pathways, especially in the production of plant growth regulators (Robinson 1974).

<u>Distribution Within the Plant</u>

Marten (1973) analyzed 12 clones of RCG for indole alkaloid concentration. Alkaloid concentrations for upper half of leaf blades, lower half of leaf blades, stems and panicles were 0.29, 0.23, 0.04, and 0.05 % dry weight, respectively. Hagman et al. (1975) also found leaf tips and leaf bases to have higher ($P<0.01$) total indole alkaloid concentration than sheaths and stems for both initial and regrowth, with the concentration in total herbage being intermediate. These researchers recommended sampling the upper one third of the crop canopy for alkaloid concentration because it more closely represented alkaloid concentration of material grazed ie. leaves.

In a study by Coulman et al. (1977b), gramine was detected largely in the leaf blades, however, hordenine was found throughout the plant, with the largest accumulations in the leaf sheaths. These authors suggested that sampling only leaf material gave an inflated value for total forage gramine concentration.

Argandona et al. (1987) found that 33 % of gramine in barley leaves was located in epidermal tissues. No gramine was detected in vascular tissues, therefore, the remaining gramine should be located in the parenchyma cells. Data has not been presented to show whether or not this is the arrangement in RCG.

Effect of Stage of Growth

Alkaloid concentration is not constant with time or stage of growth. Total indole alkaloid concentration of leaf tip and leaf base for initial growth showed a slight increase with time whereas leaf sheath and stem decreased slightly (Hagman et al. 1975). Alkaloid levels in regrowth were initially much higher than in initial growth, however, there was a large decrease in concentration with time for leaf tip, leaf base and stem. These researchers suggested that total forage alkaloid concentration for initial growth was lower than regrowth due the smaller leaf to stem ratio.

Coulman et al. (1977b) studied four clones of RCG for alkaloid concentration and observed gramine and hordenine in leaf blades to be relatively constant in initial growth. There was a large increase in the concentration of the two alkaloids of leaf blades in young regrowth but as regrowth matured the concentration decreased. Majak et al. (1979b) found levels of gramine to be elevated in regrowth but hordenine was not. The concentration of 5MeO-NMT was relatively constant throughout the growing season (<60 mg kg^{-1} dry wt). Woods et al. (1979) detected much higher levels of alkaloids in regrowth than in first growth, with hordenine content being higher in initial growth and gramine concentration higher in regrowth. Again, alkaloid concentration decreased as regrowth matured. Rapid vegetative growth ie. young regrowth seems to be a criterion for high alkaloid concentration (Woods and Clark 1971a). The grazing of RCG where regrowth does not have time to mature could lead to consumption of high alkaloid concentrations in forage.

Environmental Influences

Environment and alkaloid type interactions are small, however, interactions exist between environment and alkaloid concentration. Marten (1973) observed total indole alkaloid concentration to increase 121 % when 17 genotypes were placed under water stress. Majak et al. (1979a) compared RCG with an adequate and minimal application of water and found gramine and hordenine concentration increased ($P<0.01$) when moisture was not adequate whereas 5MeO-NMT did not. Majak et al. (1979b) recorded low alkaloid levels when RCG was grown in water saturated meadows in B.C.

Soil fertility can affect alkaloid concentration. Marten et al. (1974) found the mean total indole alkaloid concentration of four genotypes was twice as high ($P<0.05$) when grown in an infertile peat as compared to a fertile mineral soil. Parmar and Brink (1976) found increased tryptamine levels associated with the application of ammonium fertilizer as compared to nitrate sources. These authors observed that uptake of ammonium ions by RCG was greater on gleysoic soils as compared to well drained soils. Majak et al. (1979a) found the effects of nitrogen fertilizer on gramine, hordenine and 5MeO-NMT were minimized when soil moisture was adequate and the alkaloid concentrations were increased under moisture stress with the addition of nitrogen.

Toxicity

Minimal work has been carried out to gain an understanding of the toxicity of the alkaloids to the animal. Gallagher et al. (1964) injected 0.1, 0.5, and 2.0 mg 5MeO-DMT kg^{-1} body weight into sheep. The injected rate of 0.1 mg kg^{-1} would correspond to a forage level of 3 mg g^{-1} dry matter and this assumes complete absorption. This level maybe possible but the highest recorded concentration in RCG was 0.196 mg g^{-1}

and that was for 5MeO-NMT. At the low rate, sheep exhibited hyperexcitability, urination, incoordinated and backward movement. At the intermediate rate, the sheep exhibited similar symptoms in addition to collapse with over-extended legs or leg paddling but animals would recover within an hour. At the high rate, sheep collapsed with tetanic spasms, difficult breathing and eventually died due to cardiac arrest. The symptoms would occur in 13-16 seconds of injection while the oral dose took six minutes and slightly higher concentrations.

Gallagher et al. (1964) found that 5MeO-DMT was more effective than DMT to induce these effects. These dimethylated tryptamines interfere with the catabolism of serotonin (5-hydroxy tryptamine) to an inactive metabolite by competing for binding sites on monamine oxidase due to their similar structures. Serotonin functions in contraction of the smooth muscle, influences cardiac activity, and acts as an inhibitory transmitter in the parasympathetic system. If monoamine oxidase is metabolizing 5MeO-DMT and/or DMT, there will be elevated levels of serotonin, which would cause the disorder. They also suggested that the dimethylated tryptamines could interfere with the pharmacological affects of serotonin.

Ho et al. (1968) found that the β-carbolines also inhibit monoamine oxidase. It would seem likely that the β-carbolines would inhibit the enzyme rather than compete for binding sites due to the difference in structures (3 verses 2 rings).

Differences in sensitivity to alkaloid action on serotonin have been exhibited between sheep and cattle. Bovine pulmonary emphysema is exhibited in cattle but not in sheep due to differences in sensitivity of the pulmonary vein of the two species to serotonin and tryptamine

(Eyre 1975).

Gramine can have an effect on rumen microbial activity but levels of 5 mg gramine g^{-1} forage dry matter are needed (Arnold et al. 1980). This level of gramine can occur in RCG but not on a regular basis. Gramine has also been observed to impair kidney function in meadow voles by reducing the number of functional epithelial cells in the renal proximal convoluted tubule (Goelz et al. 1980). Hordenine exhibited no observable toxicity to voles, therefore seems to be much less potent.

Palatability

Total indole alkaloid concentration is negatively correlated with palatability, a measure of animal preference between feeds. Marten and Donker (1968) found heifers exhibited preference for bromegrass over RCG when grazed side by side. When restricted to one species, preference can not be exhibited therefore RCG should be grazed in monoculture. Simons and Marten (1971) found that the higher the alkaloid concentration, the less readily the plant was consumed. The correlation coefficient was 95 % for the 18 clones they evaluated with sheep. The measurement of total indole alkaloid concentration is efficient for determining unpalatable genotypes from palatable genotypes, however, the relationship is not clear for ranking the palatable genotypes. Other factors such as fiber components may cause the lack of correlation within the palatable range of genotypes.

Williams et al. (1971) also found that palatability increased with decreasing crude alkaloid content for four genotypes. They quantified gramine, hordenine, DMT, NMT, 5MeO-DMT, and 5MeO-NMT and observed 5MeO-DMT to be 18 times higher in unpalatable genotypes. No relationship was found for the other alkaloids quantified. This is of interest because

5MeO-DMT was found to be the most potent of the tryptamine derivatives tested on sheep by Gallagher et al. (1964).

Palatability is only a problem when RCG is grazed with more palatable forage species. If RCG is grazed in monoculture, palatability is not a problem unless the alkaloid concentration reaches levels that caused a reduction in intake.

Intake

Marten et al. (1976) grazed sheep on either high or low total indole alkaloid concentration RCG pasture and determined that the former pasture had lower ($P<0.05$) intake as determined by the percent of initial grass remaining seven days into the trial. This method of determining intake maybe subject to error because animals may take longer to adjust on high alkaloid pasture and growth rates of the two maybe different.

Arnold et al. (1980) observed the intake of sheep to be reduced ($P<0.001$) by adding synthetic gramine to a pelleted mixture of chopped oats and alfalfa. The concentration needed to cause a reduction ($P<0.001$) in intake was at least 5 mg gramine g^{-1} dry matter which can occur in RCG but not on a regular basis.

It has long been held that alkaloids reduce intake but data to prove this directly has not been presented. Alkaloids will inhibit intake at extreme concentrations but under commercial conditions such concentrations would not regularly occur or would only occur for a short period of time.

Digestibility

Addition of hordenine, gramine, 5MeO-DMT, and NMT to RCG substrate had no consistent effect of rumen microflora invitro with each alkaloid concentration in forage reaching 6.8 mg, 11.4 mg, 1.1 mg and 1.0 mg g^{-1} dry matter, respectively (Coulman et al. 1977a). These concentrations would be the upper limit of alkaloid concentrations in RCG. Selected clones analyzed for IVDDM showed no relationship to alkaloid content. However, Arnold et al. (1980) found gramine added to orchardgrass at concentrations equal to or greater than 10 mg g^{-1} reduced ($P<0.01$) in vitro DMD.

Animal Weight Gains

The main reason RCG has had little producer acceptance is due to the problem of animal productivity. Marten et al. (1976) monitered weight gains of lambs (2 seasons) and steers (1 season) grazing RCG. Animals were assigned to paddocks containing either genotypes of TC free or TC containing RCG material. In addition, paddocks were classified as either high or low alkaloid concentration. It was observed that animals grazing RCG with high total indole alkaloid concentration had lower ($P<0.05$) weight gains than animals grazing RCG with low alkaloid concentration. The presence or absence of the TC alkaloids in the RCG forage influenced animal weight gains on a much smaller scale than total indole alkaloid concentration.

Marten and Donker (1968) compared RCG and smooth bromegrass pasture for four years and found individual weight gains of heifers were not affected ($P>0.05$) by grass species. However, animal weight gain $acre^{-1}$ was better ($P<0.05$) with RCG due to its higher yield.

Marten et al. (1981) conducted a study to compare weight gains of lambs grazing three cultivars of RCG. The cultivars were Rise, Vantage and Mn-76. Rise was a cultivar which contained the TC alkaloids. Both Vantage and Mn-76 were free of the TC alkaloids with Mn-76 having reduced gramine levels as compared to Vantage. In year one, the presence of TC in RCG forage did not have an effect ($P<0.05$) because Rise and Vantage had similar total alkaloid concentration and lambs grazing the two cultivars had similar weight gains. The reduced ($P<0.05$) total indole alkaloid concentrations of Mn-76 resulted in higher ($P<0.05$) weight gains for grazing lambs. In the second year, total alkaloid concentration decreased ($P<0.05$) for Vantage and lamb gains on this pasture increased ($P<0.05$). These researchers suggested that lambs grazing RCG with a total indole alkaloid concentration above 2.0 mg g^{-1} will show reduced gains.

Donker et al. (1976) found that drying RCG forage reduced the alkaloid content as compared to fresh RCG and increased weight gains for sheep. Average daily gains were 48 g and -7 g for sheep fed dried and fresh cut RCG, respectively. Total indole alkaloid concentrations for the dried and fresh cut RCG forage were 1.3 and 2.2 mg/g, respectively. However, sheep gains on either dried or fresh cut RCG were still lower than sheep gains on alfalfa. Values for average daily gain of sheep fed dried and fresh cut alfalfa were 97 and 63 g, respectively. For animal gains, total indole alkaloid concentration seems to be the determining factor. The influence of individual alkaloids on animal productivity has not yet been evaluated.

Alkaloid Induced Diarrhea

The incidence of diarrhea, or an increase in moisture content of the feces, tends to increase with animals grazing RCG. Marten et al. (1981) suggested that it was the presence of TC alkaloids which caused the diarrhea. In their trial, there was a higher ($P<0.05$) incidence of diarrhea in sheep grazing Rise (TC containing) than Vantage (TC free) even though both had similar total indole alkaloid concentrations. This maybe due to a reduction in serotonin catabolism because serotonin is involved in control of smooth muscle. The diarrhea tends to be more prevalent in sheep than cattle.

Bovine Pulmonary Emphysema

Bovine pulmonary emphysema (BPE) is a disease characterized by shallow rapid respiration with difficult expiration (Carlson et al. 1968). Parmar and Brink (1976) found that BPE occurred in British Columbia when cattle were brought down from dry range to lowland meadows. The lowland meadows where pulmonary emphysema was found to occur contained RCG with tryptamines. In sheep and cattle, the pulmonary vein is much more sensitive to serotonin than the pulmonary artery. In cattle, serotonin causes constriction of the pulmonary vein. If there was a slight increase in serotonin level, such as if monoamine oxidase was inhibited, pressure would increase in the capillary beds of the lungs. This would force fluid into the interstitial spaces causing the emphysema and edema (Eyre 1975). In sheep, serotonin causes relaxation of the pulmonary vein, therefore sheep are not as susceptible to pulmonary emphysema.

STUDY I:

ALKALOID CONTENT OF REED CANARYGRASS (*Phalaris arundinaceae* L.) AS DETERMINED BY GAS-LIQUID CHROMATOGRAPHY

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Short title: Alkaloid quantification in reed canarygrass

Key Words: Gramine, hordenine, 5-methoxy-N,N-dimethyl tryptamine, reed canarygrass, *Phalaris arundinaceae* L.

ABSTRACT

A technique was developed for the quantification of gramine, hordenine and 5-methoxy-N,N-dimethyl tryptamine (5MeO-DMT) in reed canarygrass (RCG) using gas-liquid chromatography. One gram of freeze dried, ground RCG sample was extracted with methanol containing 1% ammonium hydroxide. Following solvent evaporation, the residue was extracted with dilute hydrochloric acid (0.01 N). The pH of the extract was then raised and stabilized by adding 0.5 M sodium tetraborate-boric acid buffer pH 9. The internal standard solution (0.25 mg ml^{-1}) n-nonadecane was added and the extract was purified by partitioning with chloroform. The chloroform was then evaporated and the alkaloids were derivatized with bis(trimethylsilyl)-trifluoroacetamide and effectively separated on a gas chromatographic column packed with 2% OV-7 on Chromosorb W. Derivatization was effective at 100°C (20 min). Extraction of the sample with methanol was maximal at 30 minute extraction time and the recovery of all the alkaloids studied was shown to average 90%. Development of this procedure provides a means to measure RCG gramine, hordenine and 5MeO-DMT concentrations in the presence or absence of tryptamines or β-carbolines. Initial growth and regrowth of five cultivars of RCG from two locations were evaluated for gramine, hordenine and 5MeO-DMT content using this technique. No differences were detected in hordenine content. Gramine content was higher ($P<0.05$) in Rival than Venture with Castor, Palaton and Vantage being intermediate. None of the cultivars contained detectable levels of 5MeO-DMT. There were no differences ($P>0.05$) in hordenine content between initial growth and regrowth, however, gramine content of regrowth was higher ($P<0.05$) than initial growth.

INTRODUCTION

The alkaloids of reed canarygrass (Phalaris arundincaeae L.) have been of interest due to their effect on ruminant performance (Arnold et al. 1980; Marten et al. 1981). Nine alkaloids have been detected in reed canarygrass (RCG): gramine, hordenine, four tryptamines and three β-carbolines (Marum et al. 1979). Hordenine, a phenol, is the only alkaloid detected in RCG which is not an indole alkaloid. Plant genotypes of RCG can be classified into three phenotypic groups based on the type of alkaloids present. The MeO phenotype contains the methoxy derivatives of the tryptamines and β-carbolines and the T phenotype contains the non-methoxy derivatives of the tryptamines and β-carbolines. The third phenotype, G, contains gramine and is recessive to the other two phenotypes. Hordenine does not fall into this model because of its phenolic nature (Marum et al. 1979). Most of the recently developed cultivars of RCG are of the G phenotype and also contain hordenine.

The ability to evaluate different cultivars of RCG for alkaloid content is required to understand animal productivity on the grass. Initially, total indole alkaloid concentration was used as a quantification technique (Simons and Marten 1971), however, this gave no indication as to the quantity of individual alkaloids. In addition, the extraction techniques used had a low recovery of hordenine. Colorimetric determination of gramine and hordenine was reported by Coulman et al. (1977b) and Woods et al. (1979), but only in RCG material of the G phenotype. Some of the indole alkaloids have been quantified with thin layer chromatography using the fluorescence characteristics of each compound (Majak et al. 1979b). Gas-liquid chromatography (GLC) has been used to identify the alkaloids present in RCG (Audette et al. 1969; Williams et al. 1971; Gander et al. 1976) but has not been used as a quantitative procedure.

The objective of this research was to develop a more precise and reliable chromatographic technique to quantify gramine, hordenine and 5-methoxy-N,N-dimethyl tryptamine (5MeO-DMT) in RCG. Gas-liquid chromatography was selected for the study. Alkaloids were measured in initial growth and regrowth material of five RCG cultivars free from or containing β-carbolines and tryptamines.

MATERIALS AND METHODS

Materials

Reference compounds of hordenine, gramine and 5MeO-DMT were obtained from Sigma (St. Louis, MO). The internal standard, n-nonadecane, was purchased from Chromatographic Specialities (Brockville, ON). Pyridine and bis(trimethylsilyl)-trifluoroacetamide (BSFTA) were obtained from Pierce (Rockford, IL).

Samples of RCG representing two locations in Manitoba Location 1: St. Claude on St. Claude black silty loam and Location 2: Arborg on Framnes dark wooded clay and two stages of growth were analyzed for alkaloid content using the proposed procedure. The two stages of growth, initial growth and regrowth, were cut on June 9 and August 3 at Location 1 and June 10 and July 28 at Location 2, respectively. For each location, samples were taken from two replications of a forage yield and adaptation test containing five cultivars: Castor, Palaton, Rival, Vantage and Venture. A subsample of the forage harvested from each plot, representing whole plant material, was taken and stored at -20°C.

Gas Chromatographic Analysis of Alkaloids

Extraction was performed according to Simons and Marten (1971) with modifications. Freeze dried and ground (0.5 mm screen) samples were weighed (1 g) into 125 ml flasks and shaken with 100 ml of methanol containing 1% ammonium hydroxide in an orbital shaker at 200 rpm for 30 min. Following extraction, the solution was decanted and the extract filtered (Whatman #541 paper) into a 200 ml volumetric flask. The residue was rinsed twice with a total of 90 ml of the methanol and ammonium hydroxide mixture, filtered into the volumetric flask and the final volume brought to 200 ml. A 20 ml aliquot of the crude extract was transferred into a 100 ml round bottom flask and evaporated to dryness under vacuum. The residue was then extracted twice with a total of 20 ml of 0.01 N HCl and the extract filtered (Whatman #541 paper) into a 125 ml separatory cylinder. The pH was adjusted to approximately 9 by adding 7 drops of ammonium hydroxide and then stabilized by adding 10 ml of a 0.5 M sodium tetraborate-boric acid buffer pH 9. One ml of a chloroform:methanol solution (3:1 v/v) containing the internal standard (0.25 mg ml^{-1} n-nonadecane) was added followed by 50 ml chloroform. The cylinders were shaken for 20 min. The chloroform was removed and filtered (Whatman #3 paper) into a 200 ml round bottom flask. The remaining aqueous phase was again extracted with chloroform (twice). The combined chloroform extract was concentrated under vacuum to approximately 1 ml and transferred by Pasteur pipette to a 4 ml vial.

Following drying under a nitrogen stream, 100 µl of pyridine and 100 µl BSFTA were added to the vial, which was then capped, shaken and heated for 20 min at 100°C. The trimethylsilyl derivatives of the alkaloids were separated with a Varian Vista 600 gas chromatograph (Varian, Walnut Creek, CA) equipped with a flame ionization detector and a Vista 400 computer. A glass column (1.2 x 2 mm ID) packed with 2% OV-7 on Chromosorb W, AW-DMCS (100-120 mesh; Chromatographic Specialities Inc., Brockville, ON) was used with a helium gas flow rate of 40 ml min^{-1}. The oven temperature was increased from 110°C to 230°C at a rate of 5°C min^{-1}. Injection port and detector temperatures were 260°C and 300°C, respectively.

<u>Recovery Studies and Determination of Relative Response Factors</u>

A known amount (0.25 mg) of each reference compound dissolved in a chloroform:methanol mixture (3:1 v/v) was added to a 1 g sample of freeze-dried ground timothy forage, which contained none of the alkaloids being studied. Five recovery experiments were conducted according to the procedure described above. The alkaloids were measured based on relative response factors, which were determined with the response for n-nonadecane set at 1.00. Relative response factors were used to compensate for differences in the flame-ionization detector response for each alkaloid relative to n-nonadecane (A, Table 1). However, for alkaloid determination in the RCG samples the response factors were determined differently. A known amount of the reference compounds were subjected to all steps of extraction and purification so as to compensate not only for differences in detector response but also for incomplete recovery of alkaloids in the procedure (B, Table 1).

Statistical Analysis

Results were analyzed as a split-plot design with location as the main effect, and stage of growth and cultivar as sub effects. The general linear models procedure from (SAS Institute Inc. 1985) was used to analyze data with replications within location as the error term for location. The interaction of stage of growth and replication effects within location was used as the error term for stage of growth. The interaction of cultivar and replication effects within location effects was used as the error term for cultivar. Mean separations were performed using Bonferoni's Inequalities Test with the appropriate error term.

Table 1: Relative response factors for quantification of alkaloids with n-nonadecane as internal standard

Alkaloid	Relative Response Factors A†	Relative Response Factors B‡
Hordenine	1.37	1.60
Gramine	1.16	1.30
5-methoxy-N,N-dimethyl tryptamine	1.27	1.34

† Represents differences in detector response.
‡ Represents the sum of differences in detector response and incomplete recovery of alkaloids.

RESULTS AND DISCUSSION

Derivatization was shown to be necessary for optimum separation of the alkaloid compounds. Both gramine and hordenine could be readily derivatized with BSFTA at room temperature but 5MeO-DMT required higher temperatures (100°C) and prolonged time (20 min) for derivatization. The trimethylsilyl derivatives of all three alkaloids were well separated (Figure 1) with no interference from impurities in a run time of 24 minutes.

Extraction times of forage samples in methanol and ammonium hydroxide were tested from 5 to 120 minutes (Table 2). Recovery of alkaloids improved from 5 to 30 minutes of extraction time with little or no change with longer extraction times. Consequently, an extraction time of 30 minutes was adopted as the standard procedure.

The form of RCG material being extracted had an effect on the precision of the alkaloid analysis (Table 3). Initially, material was frozen and chopped, however, this resulted in a wide range of values for repeated determinations of the alkaloids from the same forage sample. Chopped material was not sufficiently homogenous to allow 1 g DM of sample to be representative due to variations in alkaloid content within the plant. Freeze drying and grinding resulted in a more uniform sample, reducing the variation between readings of alkaloid content from the same sample. It was found that purification of the crude extract by partitioning the aqueous solution with chloroform resulted in a low recovery of hordenine. The use of a buffer to stabilize the pH at 9 as well as prolonged extraction with chloroform (3 times of 20 minutes each) improved the recovery of hordenine as well as the other alkaloids studied. A comparison of alkaloid recoveries using the proposed

Figure 1. Gas chromatogram of trimethylsilyl derivatives of alkaloids from a mixture of pure compounds added (0.25 mg g^{-1} DM) to a sample of timothy (A) and naturally occuring compounds in a sample of reed canarygrass (B). Peaks: 1 = hordenine; 2 = gramine; 3 = n-nonadecane (internal standard); 4 = 5-methoxy-N,N-dimethyl tryptamine.

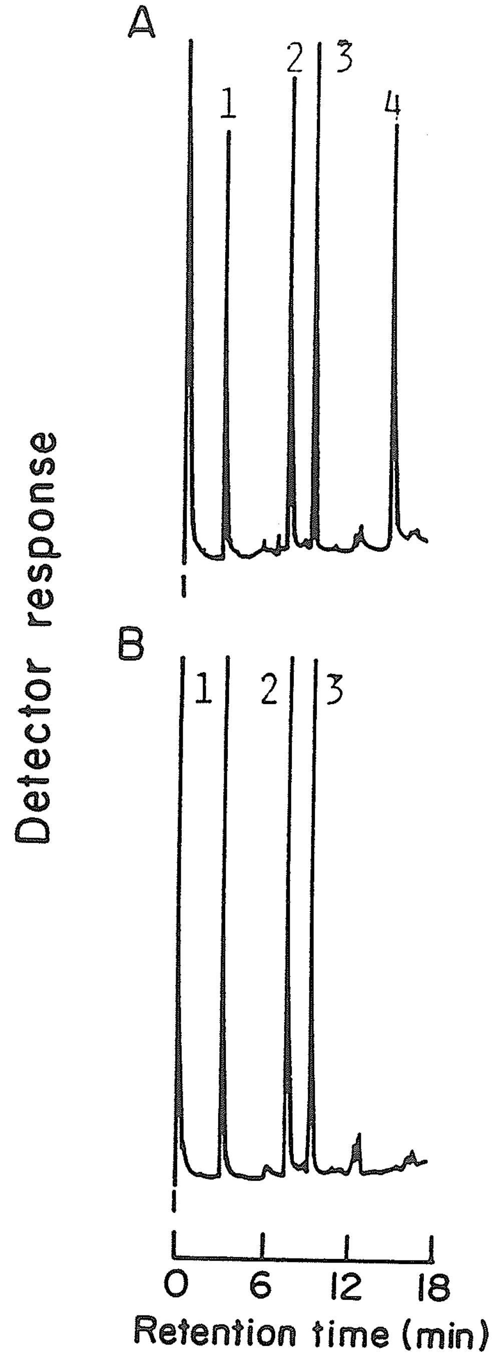
A
1
2
3
4
B
1
2
3
Detector response
0
6
12
18
Retention time (min)

Table 2: Effect of varying times of extraction on the recovery of alkaloids from reed canarygrass

Extraction time (min)	Recovery (mg g^{-1} DM)
5	4.5 ± 0.5†
15	5.0 ± 0.3
30	5.8 ± 0.2
60	5.8 ± 0.2
120	5.8 ± 0.2

† Mean ± SD

Table 3: Effect of freeze-drying and grinding of the reed canarygrass sample on the precision of alkaloid analysis

Sample form	Total alkaloids (mg g^{-1} DM)†	
	Mean ± SD	Range
Frozen, chopped material	5.7 ± 1.1	4.2 - 7.6
Freeze-dried, ground material	5.8 ± 0.2	5.7 - 6.1

† Includes hordenine, gramine and 5-methoxy-N,N-dimethyl tryptamine.

technique with the modified purification procedures and that of Simons and Marten (1971) is given in Table 4.

Castor, a cultivar of the MeO phenotype, has been shown to contain 5-methoxy-N-methyltryptamine (Majak et al. 1979b) whereas the other four cultivars are of the G phenotype. 5MeO-DMT was not detected in any of the Castor samples, suggesting that 5MeO-DMT was not present in Castor or was present at levels below detection. Majak et al. (1979b) detected 5MeO-DMT in Castor samples grown in a growth cabinet but not when grown under field conditions.

There were no differences ($P>0.05$) observed between cultivars for hordenine concentration for the two locations and stages of growth (Table 5). However, Rival and Castor tended to be higher than Venture ($P=0.12$). Differences were observed among cultivars for gramine concentration. Rival had a higher ($P<0.05$) concentration of gramine than Venture, with the remaining cultivars being intermediate.

There were no differences ($P>0.05$) between locations for either gramine or hordenine concentration (Table 6). There was an interaction ($P<0.05$) between cultivar and location for gramine concentration. Gramine concentrations for Rival and Venture were higher at Location 1 than Location 2 whereas Palaton showed the opposite response and Castor and Vantage had similar values at both locations.

Effect of stage of growth on alkaloid concentration was tested. Hordenine concentration did not differ ($P>0.05$) between the two stages of growth whereas gramine concentration was lower ($P<0.05$) for initial growth than regrowth (Table 7). This latter relationship is consistent with previous research (Coulman et al. 1977b).

Prior to analysis of samples, it was noticed that one of the

Table 4: Recovery (%) of alkaloids as determined by gas-liquid chromatography using two different procedures of extraction

Extraction procedure	Recovery		
	Hordenine	Gramine	5MeO-DMT†
Simons and Marten method	21.8 ± 1.1‡	79.2 ± 4.3	88.6 ± 6.1
Proposed method	85.9 ± 2.1	89.3 ± 3.1	94.9 ± 2.9

† 5-methoxy-N,N-dimethyl tryptamine
‡ Mean ± SD

Table 5: Alkaloid concentration (mg g^{-1} DM) for five cultivars of reed canarygrass

Cultivar	Hordenine	Gramine
Rival	2.03 ± 0.11†	0.93 ± 0.06a
Castor	2.10 ± 0.14	0.76 ± 0.08ab
Vantage	1.83 ± 0.11	0.76 ± 0.06ab
Palaton	1.77 ± 0.11	0.70 ± 0.06ab
Venture	1.53 ± 0.11	0.58 ± 0.06 b

a-b Least square means in columns with different letters are different (P<0.05) using Bonferroni's inequalities test.

† Least square means and standard errors for two locations and two stages of growth.

Table 6: Effect of location on alkaloid concentration (mg g^{-1} DM) of reed canarygrass

Location	Hordenine	Gramine
1	1.94 ± 0.14†	0.70 ± 0.08
2	1.76 ± 0.15	0.77 ± 0.09

† Least square means and standard errors of two harvest dates and five cultivars.

Table 7: Effect of stage of growth on alkaloid concentration ($mg\ g^{-1}$ DM) of reed canarygrass

Stage of growth	Hordenine	Gramine
Initial growth	1.84 ± 0.07†	0.28 ± 0.04b
Regrowth	1.86 ± 0.08	1.19 ± 0.05a

a-b Least square means in columns with different letters are different (P<0.05) using Bonferroni's inequalities test.

† Least square means and standard errors of five cultivars and two locations.

regrowth Castor samples from Location 1 had not been stored properly and had evidence of heating. This sample was not included in the statistical analysis, however, it was analyzed for alkaloid content and no peaks corresponding to the alkaloids were detected. The reduced alkaloid content of RCG hay as compared to fresh forage was noted by Donker et al. (1976) and may be the result of heating that can occur during hay drying and storage.

The levels of gramine detected in these samples should not affect ruminant performance as Marten et al. (1981) determined that values of indole alkaloids below 2 mg g^{-1} DM had no effect on the ruminant animal. Hordenine, however, is not an indole alkaloid and its effect on ruminant performance needs further study.

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STUDY II:

COMPARISON OF INTAKE AND DIGESTIBILITY FOR THREE CULTIVARS OF REED CANARYGRASS (*Phalaris arundinaceae* L.) AND ONE CULTIVAR OF TIMOTHY (*Phleum pratense* L.)

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Short Title: Intake and digestibility of reed canarygrass

Key Words: Reed canarygrass, low alkaloid, gramine, hordenine, intake, digestibility

ABSTRACT

An intake and digestibility trial was conducted for initial growth and regrowth of Rival, Venture and Frontier reed canarygrass and Champ timothy. Forage of each cultivar was fresh frozen and fed to ten lambs (six female and four male) for determination of daily dry matter intake over a seven day period. The four ram lambs were then restricted to 90% of ad libitum dry matter (DM) intake for determination of forage DM, crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) apparent digestibility. Content of CP, ADF, NDF, calcium, phosphorus, magnesium, sodium, potassium, sulfur, zinc, copper, gramine and hordenine were determined for initial growth and regrowth of each cultivar. No differences ($P>0.05$) were observed among cultivars for dry matter intake of either initial growth or regrowth. There were differences ($P<0.05$) among cultivars for DM, CP, ADF and NDF digestibilities in both initial growth and regrowth. Rival and Champ had better DM digestibility than did Frontier or Venture for initial and regrowth forage material. Gramine and hordenine concentrations of RCG cultivars were low in both initial growth and regrowth, and seemed to have no affect on intake or digestibility.

INTRODUCTION

A number of agronomic characteristics, including adaptation to low lying areas and drought tolerance, has led to continued interest in the use of reed canarygrass (RCG) Phalaris arundinaceae L. as a forage crop. Commercial use of this forage crop has remained depressed, however, due in part to poor animal productivity. Low dry matter (DM) intake (Marten et al. 1976) and poor animal weight gain (Donker et al. 1976) observed in animals grazing RCG have been attributed to a group of compounds identified as alkaloids.

Alkaloids isolated in RCG include gramine, hordenine, four tryptamines and three β-carbolines. The effects of alkaloid type and concentration upon intake and digestibility of the RCG consumed by grazing animals are poorly documented. Addition of synthetic gramine to ruminant diets has been shown to decrease DM intake and in vitro DM disappearance at concentrations of 5 and 10 mg g^{-1} DM, respectively, (Arnold et al. 1980). These high concentrations of gramine, however, are rarely observed under field conditions. Marten et al. (1981) estimated that the mean threshold level for total indole alkaloids at which lamb weight gain on pasture is reduced is 2.0 mg g^{-1} DM, however, effects on intake and digestibility were not established. Several studies (Barnes and Mott 1970; Neihaus 1971) have established differences in digestibility between cultivars of RCG, however, determinations for alkaloid type or concentrations were not available.

The objective of this study was to compare intake and apparent digestibility of DM, crude protein and fiber components of initial growth and regrowth forage material from three cultivars of RCG and one of timothy. Frontier contains tryptamines and β-carboline alkaloids in addition to gramine and hordenine. Frontier was developed in Canada and licensed in 1959. Rival and Venture, two low alkaloid RCG cultivars contain only hordenine and gramine alkaloids and were licensed in 1987 and 1985, respectively. Champ, a timothy cultivar, does not contain alkaloids. Forage alkaloid content was measured to determine the possible effects of gramine and hordenine on intake and digestibility.

MATERIALS AND METHODS

Forage

One paddock (0.8 ha) of each of Rival, Venture, Frontier and Champ were used to evaluate the intake and digestibility of initial growth (Experiment 1) and regrowth (Experiment 2) of each cultivar of grass. The paddocks, located on osborne clay soil at the University of Manitoba campus, were seeded in June 1986. The paddocks were fertilized with 60 kg nitrogen ha^{-1} and 30 kg phosphate ha^{-1} applied as ammonium nitrate phosphate (34-17-0) on May 9, 1987. Broadleaf weeds were controlled with an application of 2,4-D amine (2 kg active ingredient ha^{-1}) on May 11, 1987.

The forage was cut into swaths using a 4.4 m New Holland haybine. Immediately after cutting, forage was chopped into 1-2 cm pieces and blown into a forage wagon using a New Holland forage harvester. From the wagon, forage was unloaded into burlap bags. Dry ice nuggets were added at the time of filling at a rate of 10% wt/wt. The burlap bags were closed, placed into plastic bags and moved to walk-in freezers for storage (-10 to -20°C). Forage was removed from freezers as required for the lamb trials.

Experiment 1

In experiment 1, the intake and digestibility were determined for initial growth of Rival, Venture, Frontier and Champ forage, cut and frozen on June 4, 5, 8 and 9, 1987, respectively. These cutting dates represented heading to early anthesis of the grass.

Intake of the grasses was evaluated with forty growing lambs (10 lambs/cultivar) averaging 22.4 ± 0.4 kg and 93.8 ± 0.6 days of age at the start of the experiment. Sixteen male and twenty-four female lambs were used, of which half of each sex was purebred Outaouais Arcott and the other half was crossbred Suffolk * Outaouais Arcott. Animals were balanced for breed and sex, and assigned to ensure uniform body weight across treatments.

The adjustment phase of the intake trial was from day 1 (June 2) to day 24. Lambs assigned to each of the four cultivars were group fed on an ad libitum basis until day 20. On day 21 rams were moved to raised metabolism crates and ewes to individual stalls. Enough forage could not be frozen for the entire experiment, therefore, fresh forage of each cultivar, cut just prior to feeding, was fed from day 1 to day 10. Frozen forage was used from day 11 until the end of the experiment. For the entire experiment lambs were fed twice daily at 8:00 and 16:00 h. During the intake trial (day 25 to 32), forage offered and weighback were measured daily. The quantity of forage offered was such that a 10% weighback was expected. On day 33, the intake trial was completed. Ewe lambs were removed from test and ram lamb intake was reduced to 90% of ad libitum. The digestibility trial started on day 35 and lasted 7 days. Throughout the experiment, lambs had access to water and received 50 g day^{-1} of a mineral mix containing 54.8% cobalt iodized salt, 19.8% Biofos (Pitman-Moore Inc., Mundelein, IL) and 25.4% calcium carbonate.

<u>Experiment 2</u>

Experiment 2 evaluated the intake and digestibility for regrowth of Rival, Venture, Frontier and Champ, cut and frozen on July 16, 15, 27 and 24, respectively. Regrowth of the reed canarygrass cultivars was in the 5 to 6 leaf vegetative stage when cut whereas Champ was cut at early heading. The cutting of Champ and Frontier was delayed due to excess precipitation which made it difficult to get equipment onto the paddocks.

The 40 lambs used to evaluate intake and digestibility of regrowth had the same ratio of female to male and breed groups as described for Experiment 1. The second group averaged 21.8 ± 0.3 kg body weight and 87.9 ± 0.6 days of age.

The adjustment phase, day 1 (July 7) to 30, of the intake trial was longer than for Experiment 1 because of the delay in cutting Frontier and Champ. Fresh cut material was fed until day 21, after which frozen forage for all four cultivars was offered. Lambs were fed their assigned forages in groups until day 25, after which they were moved to individual digestion crates or stalls. The intake trial was performed for a seven day period as for Experiment 1 (day 31 to 38). On day 39 ewe lambs were removed from test and ram lamb intake was reduced to 90% ad libitum for the start of the digestibility trial. The 7 day digestibility trial started on day 42. Mineral supplementation and water were provided to lambs as described for experiment 1.

Data Collection

For the intake trials, forage offered and weighback were measured on a daily basis for each lamb. Samples of each cultivar and the weighbacks were collected and stored (-20°C). At the end of the intake trials, daily forage samples and the weighbacks were dried in a forced air oven (60°C) to determine DM content and composited to result in one forage composite for each cultivar of forage offered and a weighback composite for each lamb.

For the digestibility trials, forage offered, weighback and feces collected were measured daily for each lamb. Samples of forage, weighback and feces were collected daily and stored (-20°C). At the end of the digestibility trials, daily forage samples, weighbacks and feces were dried in a forced air oven (60°C) to determine dry matter content and composited for each lamb.

For both the intake and digestibility trials, daily forage samples of each cultivar were collected, composited and stored (-20°C) for alkaloid analysis.

Chemical Analysis

Dried samples of forage, weighback and feces were ground to pass through a 1 mm screen in a Wiley mill for both experiment 1 and 2. Crude protein (CP) content was determined as N*6.25 with a Kjeltec system 1002 Distilling Unit (Fisher Scientific, Edmonton, AB) according to Association of Analytical Chemists (AOAC; 38.012; 1975). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) content was determined (Goering and Van Soest 1970) using refluxing apparatus (Laboratory Construction Co., Kansas City, MO). Concentrations of phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn) and copper (Cu) of only forage composites were determined by dry ashing a 1 g sample at 550°C for 12 hours. The P concentration was determined colorimetrically (AOAC; 7.103-7.105; 1975) using a Bausch and Lomb Spectronic 20 (Fisher Scientific, Edmonton, AB). Calcium, Mg, K, Na, Zn, and Cu content were determined (AOAC; 7.077-7.082; 1975) using flame atomic absorption (Instrument Laboratory AA/AE Spectrophotometric Model 551, Instruments laboratory Inc., Wilmington, Massachusetts). Forage sulfur (S) concentration was analyzed as described by Boila et al. (1984). Gramine and hordenine concentration were determined by gas-liquid chromatography (Duynisveld et al. 1990).

Statistical Analysis

Dry matter intake was analyzed as a split plot using analysis of variance (SAS Institute Inc. 1985) with cultivar and sex of lamb as main effects and day as sub effect. Cultivar and sex effects were tested using lamb within the interaction of cultivar and sex effects error term. Digestibilities were analyzed with analysis of variance (SAS 1985) with lambs nested within cultivars. Mean comparisons were performed using the Duncan's multiple range test and the appropriate error term.

RESULTS AND DISCUSSION

Experiment 1

The RCG cultivars had higher CP levels than Champ when all cultivars were cut at heading to early anthesis (Table 8). The ADF contents were variable for RCG cultivars, however Rival tended to be lower than Champ. The NDF content of RCG cultivars was lower than Champ with Rival tending to be the lowest. Grass species did not appear to influence mineral concentration in the forage with the exception of K, S and Cu. The concentration of K was high in Rival relative to other cultivars of RCG and Champ. Sulfur was lower for Champ than for RCG cultivars. In this experiment, Cu concentration for Champ was 3 to 5 mg g^{-1} DM higher than for RCG cultivars.

Samples of forage contained nondetectable levels of gramine (Table 8). Hordenine levels for the three cultivars of RCG were uniform. Alkaloids were not detected in Champ.

There were no differences ($P>0.05$) between cultivars for DMI expressed as either intake $animal^{-1}$ day^{-1} or percent of body weight (Table 9). Expressed as a % of body weight, DMI averaged 2.59 for Champ and 2.75 for the RCG cultivars. Therefore, it does not appear that hordenine concentrations observed in the initial growth inhibited DMI in this experiment.

Cultivar of grass did influence DM and nutrient apparent digestibility, ($P<0.05$; Table 9). Apparent DM digestibility of Champ was higher than Frontier and Venture even though ADF values were similar. Differences were also observed for DM digestibility among RCG cultivars, Frontier and Venture having lower digestibilities than Rival ($P<0.05$).

TABLE 8. Nutrient composition and alkaloid concentration, DM basis, of initial growth for each cultivar of grass.┼

	Reed Canarygrass			Timothy
	Frontier	Rival	Venture	Champ
Dry matter (%)	23.6	21.2	27.5	27.4
Crude protein (%)	17.1	18.5	16.3	13.3
Acid detergent fiber (%)	37.0	34.8	37.2	36.8
Neutral detergent fiber (%)	64.2	61.6	65.0	67.8
Calcium (g kg^{-1})	3.3	3.3	2.8	2.9
Phosphorus (g kg^{-1})	3.2	3.0	2.6	2.9
Potassium (g kg^{-1})	26.3	30.6	22.9	23.4
Sodium (g kg^{-1})	0.3	0.5	0.3	0.4
Magnesium (g kg^{-1})	3.2	3.8	2.7	2.4
Sulfur (g kg^{-1})	2.1	2.4	2.7	1.5
Copper (mg kg^{-1})	5.1	6.9	5.0	10.2
Zinc (mg kg^{-1})	20.3	25.7	23.1	24.9
Hordenine (mg g^{-1})	0.68	0.53	0.55	ND‡
Gramine (mg g^{-1})	ND	ND	ND	ND

┼ Based on analysis of a composite of frozen forage fed during the intake and digestibility trial.

‡ ND - not detected.

TABLE 9. Daily dry matter intake and apparent digestibility of initial growth for each cultivar of grass when fed to growing lambs.

	Reed Canarygrass			Timothy	
	Frontier	Rival	Venture	Champ	Standard Error
Daily Dry Matter Intake					
g d^{-1} †	659	710	746	662	48
% body weight †	2.65	2.82	2.86	2.62	0.11
Apparent Digestibility (%)					
Dry matter ‡	58.3c	60.4b	58.8c	62.3a	0.4
Crude protein ‡	73.8b	73.1b	76.8a	69.7c	0.6
Acid detergent fiber ‡	52.0b	55.6a	51.8b	57.8a	0.7
Neutral detergent fiber ‡	57.3c	62.2b	58.8c	65.8a	0.8

a-c means in rows with different letters are different ($P<0.05$).
† n=10
‡ n=4

In general, fiber digestibilities were similar to DM digestibility for each cultivar of grass. Cultivar rankings for NDF digestibility were similar to DM digestibility. In the case of ADF digestibility, Rival was not different ($P>0.05$) from Champ and higher than Venture and Frontier. Rival and Champ have higher ADF digestibilities and therefore higher DM and NDF digestibilities than Frontier and Venture.

Apparent CP digestibilities did not follow patterns observed for DM digestibilities. The RCG cultivars, which had higher CP levels, were observed to have higher CP digestibilities relative to timothy. There was some variability in CP digestibility among RCG cultivars, Venture having higher digestibility than Frontier or Rival ($P<0.05$).

Differences observed in the apparent DM and fiber digestibility between cultivars of grass did not appear to be associated with grass nutrient contents. Therefore, some non-nutrient factor maybe influencing the digestibility of these cultivars of grass.

Experiment 2

Nutrient content of cultivars in this experiment is considered to be a reflection of cultivar and maturity differences. Time interval between harvesting initial growth and regrowth of each cultivar was 42, 42, 50 and 46 days for Rival, Venture, Frontier and Champ, respectively. As for initial growth, the CP level for Champ was lower than for the RCG cultivars (Table 10). It is not certain whether the differences between Frontier and the other RCG cultivars is related to cultivar or maturity of the regrowth. The ADF and NDF contents were higher for Frontier and Champ than for the other cultivars. As was observed in experiment 1, K, S and Cu differed in concentration between cultivars, with trends for all three being similar to initial growth. Forage Ca concentrations

TABLE 10. Nutrient composition and alkaloid concentration, DM basis, of regrowth for each cultivar of grass.†

	Reed Canarygrass			Timothy
	Frontier	Rival	Venture	Champ
Dry matter (%)	20.2	19.7	21.6	23.5
Crude protein (%)	14.2	18.9	18.3	12.3
Acid detergent fiber (%)	41.6	37.4	37.8	40.8
Neutral detergent fiber (%)	66.5	62.3	64.7	67.5
Calcium (g kg^{-1})	3.0	3.7	4.3	3.4
Phosphorus (g kg^{-1})	3.7	3.5	3.5	3.1
Potassium (g kg^{-1})	27.3	32.1	26.9	24.2
Sodium (g kg^{-1})	0.3	0.5	0.4	0.3
Magnesium (g kg^{-1})	3.2	4.4	3.8	2.4
Sulfur (g kg^{-1})	2.3	2.8	2.8	1.4
Copper (mg kg^{-1})	4.3	4.9	4.9	7.8
Zinc (mg kg^{-1})	18.3	23.8	25.8	21.5
Hordenine (mg g^{-1})	1.41	1.70	1.31	ND‡
Gramine (mg g^{-1})	0.52	1.23	0.55	ND

† Based on analysis of a composite of frozen grass fed during the intake and digestibility trial.

‡ ND - not detected.

were high for Rival and Venture relative to Frontier. Concentrations of hordenine and gramine for RCG cultivars were higher for regrowth than for initial growth forage material (Table 10). Rival tended to be higher than the other two RCG cultivars for both gramine and hordenine.

Dry matter intake was not influenced by cultivar (Table 11). Average DMI, expressed as % of body weight, were 2.52 for Champ and 2.59 for the RCG cultivars. Although both gramine and hordenine content in Rival (1.23 and 1.70 mg g^{-1} DM, respectively) was higher than in Venture (0.55 and 1.31 mg g^{-1} DM, respectively), no differences in intake were observed between these two cultivars. Higher fiber concentrations in Champ and Frontier forage material as compared to Rival and Venture make comparison based on total absence of alkaloids or the presence of tryptamines and β-carbolines difficult.

Differences in apparent DM digestibility for cultivars of grass did not reflect differences observed for ADF content nor alkaloid content (Table 11). Champ had DM digestibilities similar to Rival and higher ($P<0.05$) than Frontier and Venture. As for experiment 1, forage CP content and CP digestibility were positively related. Apparent digestibility of ADF was greater for Rival than for the other three cultivars of grass. The NDF digestibility rankings were similar to that observed for DM digestibility.

Despite a higher fiber concentration, apparent DM and fiber digestibility for Champ was high relative to the RCG cultivars, with the exception of Rival. Dry matter and nutrient digestibility of Rival was superior to other RCG cultivars and similar or superior to that observed for Champ when forage material was cut at similar stages of maturity. Therefore, it should be assumed that alkaloid concentration in forage

TABLE 11. Daily dry matter intake and apparent digestibility of regrowth for each cultivar of grass when fed to growing lambs.

	Reed Canarygrass			Timothy	Standard Error
	Frontier	Rival	Venture	Champ	
Daily Dry Matter Intake					
$g\ d^{-1}$ †	613	642	616	644	44
% body weight †	2.52	2.66	2.59	2.52	0.13
Apparent Digestibility (%)					
Dry matter ‡	54.4b	60.7a	55.2b	58.5a	1.4
Crude protein ‡	72.9b	76.2a	73.4b	63.8c	0.8
Acid detergent fiber ‡	49.4b	56.2a	47.9b	50.1b	1.1
Neutral detergent fiber ‡	54.6b	61.4a	54.7b	59.1a	1.3

a-c means in rows with different letters are different ($P<0.05$).
† n=10
‡ n=4

regrowth in this experiment did not adversely affect digestibility or intake because hordenine and gramine alkaloid levels were highest in Rival.

CONCLUSION

Cultivar of RCG did not influence DM intake for initial growth or regrowth when grasses were harvested at a similar stage of maturity. Intake differences were not detected between RCG and Champ timothy. Apparent DM and fiber digestibility of Rival initial growth and regrowth forage material was higher than for Frontier and Venture. Apparent DM and fiber digestibility for Champ initial and regrowth forage material was superior or equal to Rival despite lower forage fiber concentrations for the latter. Alkaloid type and concentration in forage material did not adversely influence intake. Although Rival regrowth material had the highest gramine and hordenine concentrations, it also had the highest digestibility values suggesting that alkaloid concentrations equal to or lower than those tested in this study will not adversely affect DM or nutrient digestibility.

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STUDY III:

EVALUATION OF RIVAL, VENTURE AND FRONTIER REED CANARYGRASS (*Phalaris arundinaceae* L.) AS PASTURE FORAGE UNDER DROUGHT CONDITIONS

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Short Title: Reed Canarygrass as Pasture Forage

Key Words: Reed canarygrass, pasture, gramine, hordenine

ABSTRACT

A grazing study was conducted to determine the effect of cultivar of reed canarygrass (RCG) on productivity of the grazing animal. Two (0.5 ha) paddocks of three RCG cultivars identified as Rival, Venture and Frontier and one cultivar of timothy, Champ, were used in the study. The weight gains of ten lambs in each paddock were monitored from May 25 to July 5, period I, and July 20 to August 30, period II. Crude protein, acid detergent fiber, neutral detergent fiber, gramine and hordenine concentrations in forage were determined weekly. Calcium, phosphorus, magnesium, sulfur, copper and zinc were determined at three dates during each period. Hot dry conditions experienced during both grazing periods resulted in the removal of the lambs grazing in the Champ paddocks due to inadequate forage production. There were no differences ($P>0.05$) in nutrient and alkaloid composition between cultivars of RCG in period I whereas in period II there were differences ($P<0.05$) in gramine and fiber concentrations. Differences ($P=0.07$) were detected among cultivars for average daily gains (ADG) of lambs: means (± SE) were 119, 146 and 161 ± 8 g for Frontier, Rival and Venture, respectively. An interaction ($P=0.06$) for lamb ADG between cultivar and period reflected changes in forage alkaloid concentration under drought conditions. Seven lambs from each cultivar, placed on a 56 day finishing ration following period II, did not exhibit any residual or carryover effects due to the extended exposure to gramine or hordenine.

INTRODUCTION

Reed canarygrass (RCG), Phalaris arundinaceae L., has potential as a pasture grass due to a good distribution of growth from early spring to late summer, high nutrient quality, high yield potential, flooding and drought tolerance and regrowth ability. However, performance of ruminants grazing RCG maybe poor due to the presence of alkaloid compounds. Alkaloids which maybe detected in RCG include gramine, hordenine, four tryptamines and three β-carbolines. Marten et al. (1976) determined that total indole alkaloid concentration (gramine, tryptamines and β-carbolines) not individual alkaloid concentration was inversely correlated with gains of lambs and steers. Marten et al. (1981) suggested that total indole alkaloid concentrations above 2 mg g^{-1} dry weight of forage will reduce animal gains on pasture. Palatability (valued at 10=rejected, 0=consumed) between 18 clones of RCG has been shown to be correlated (r=+.95) with total indole alkaloid concentration (Simons and Marten 1971). The presence of tryptamine and β-carboline alkaloids can cause diarrhea in animals grazing RCG (Marten et al. 1976, 1981).

Alkaloids detected in RCG have been used to classify various plant genotypes into three phenotypic groups: the MeO phenotype containing methoxy derivatives of the tryptamines and β-carbolines, the T phenotype containing non-methoxy derivatives of tryptamines and β-carbolines and the G phenotype containing gramine (Marum et al. 1979). Hordenine is a major alkaloid of RCG which does not fall into any category of this model because of its phenolic structure. Many of the recently released RCG cultivars are of the G phenotype, containing only gramine and hordenine.

The objective of this research was to gain an insight into the effect of RCG cultivars varing in alkaloid type and concentration on the productivity of the grazing animal. Two cultivars of the G phenotype were used: Rival and Venture. Frontier, a tryptamine and β-carboline containing cultivar of the MeO phenotype was also incorporated into this study. These three cultivars were to be compared with Champ timothy, a non-alkaloid containing grass. The grasses were continuously grazed to provide conditions of elevated alkaloid concentration (Woods and Clark 1971a). Following the grazing trial, the animals were placed on a finishing ration to determine if there were residual effects of long term exposure to the alkaloids on animal productivity.

MATERIALS AND METHODS

Paddocks

Eight paddocks (0.5 ha) seeded to one of Rival (2 paddocks), Venture (2 paddocks), Frontier (2 paddocks) or Champ (2 paddocks) were used in the grazing study. Paddocks were initially seeded in June 1986 and portions reseeded in May 1987. Paddocks were located on Osborne clay soil at the University of Manitoba campus.

Pasture fertilization was based on results from soil samples taken during the fall of 1987. Spring (April 7, 1988) application was conducted to ensure that all pastures had a calculated soil content of 70 kg nitrate-N and 40 kg phosphate ha^{-1}. Ammonium nitrate and ammonium phosphate were used as sources of nitrogen and/or phosphate. A rotary mower was used to remove any standing stubble on April 14, 1988.

A put and take system, using yearling and mature ewes from the University of Manitoba research flock, was employed for the duration of the pasture study to ensure that forage in all paddocks was maintained in a vegetative state. All animals were provided with a constant supply of fresh water, shade and cobalt iodized salt.

Four movable chain link exculsion cages (2.5 x 1.0 m) were placed in each paddock and forage samples (1.0 x 0.5 m) were hand cut from a site within each cage on a weekly basis. Cages were randomly relocated following each sampling. Forage samples taken in each paddock were composited and stored (-20°C).

Animals

Crossbred lambs averaging 63.0 ± 0.4 days of age and 18.0 ± 0.3 kg body weight were used in the pasture study. Dams for all lambs were a Suffolk * Outaouais Arcott crossbred. Sire breed of lambs were either

Hampshire (2 lambs paddock^{-1}) or Canadian Arcott (8 lambs paddock^{-1}). Five intact ram and five ewe lambs were assigned to each of the paddocks.

Lambs were placed on pasture in two groups. The first group, consisting of 3 ram and 2 ewe lambs paddock^{-1}, were placed into their assigned paddocks on May 20. The second group of lambs, consisting of 2 ram and 3 ewe lambs, were placed on pasture June 3. Lambs were grouped in order to assure uniform age and body weight at the time they were placed on pasture. All lambs were removed from the test paddocks on July 5 because of reduced pasture growth, which was a result of minimal precipitation. Lambs assigned to the RCG paddocks were maintained on adjacent paddocks of the same cultivar for a two week period, and then returned to their assigned paddocks on July 19, remaining there until the end of the trial, August 30. Poor growth in the paddocks seeded to Champ timothy required that these lambs be placed on non-experimental grass pasture from June 21 through to the end of the grazing season. It was decided to continue monitoring body weights of lambs initially assigned to Champ for comparison with lambs assigned to the RCG cultivars.

Lamb body weights were measured at biweekly intervals during the grazing season. Initial body weight measurements were taken five days after lambs were placed on pasture to allow for adjustment in body fill. The last day of each period coincided with the last biweekly weighing of the lambs. Since animals were removed from their assigned paddocks for a two week period, average daily gain comparisons were based on data collected prior to July 5 (Period I) and after July 19 (Period II). Observations for the symptoms of diarrhea in lambs for all paddocks were made on June 24 and July 20. Animal grazing days ha^{-1} for each paddock were monitored for period I and II, and determined using coefficients of 0.07, 0.11, and 0.14 animal unit grazing days for lambs, yearling ewes and mature ewes, respectively (Ensminger 1970).

Finishing Trial

Seven lambs originally assigned to each of the three RCG cultivars and to Champ were selected on the basis of body weight for this trial. Inorder to reduce animal varibility due to sex and breed, only ram lambs sired by Outaouais were selected.

A pelleted ground barley grain-alfalfa meal ration (Table 12) was fed to lambs on an ad libitum basis using self feeders. The animals were allowed a 12 day period to adjust from pasture forage to pelleted diet prior to the start of the trial. Fresh water and straw was available at all times. All lambs were housed in one feedlot.

Animal weight measurements were taken on two consecutive days at the beginning and end of the trial and at biweekly intervals during the 56 day trial.

Chemical Analysis

Forage composite samples were subsampled and the subsample was stored at (-20°C). The remainder of the composite sample was dried in a forced air oven (60°C) for dry matter determination and ground through a 1.0 mm screen in a Wiley mill for further chemical analyses. Crude protein (CP) content was determined as N*6.25 with a Tecator Auto 1030 Analyzer (Fisher Scientific, Edmonton, AB) according to Association of Analytical Chemists (AOAC; 38.012; 1965). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined (Goering and Van Soest, 1970) using a Tecator Fibertec System 1020 Hot Extractor (Fisher

TABLE 12. Ingredient and chemical composition, DM basis, of the finishing ration fed to ram lambs.

Ingredients	%
Crushed barley	76.5
Dehydrated alfalfa meal	18.5
Sugar beet molasses	1.7
Limestone	1.7
Cobalt iodized salt	0.6
Vitamin premix†	1.0
Chemical Composition	
Crude protein (%)	13.5
Acid detergent fiber (%)	10.6
Neutral detergent fiber (%)	21.5
Calcium (g kg^{-1})	1.03
Phosphorus (g kg^{-1})	0.30

† Vitamin premix formulated to contain 63,800 IU Vitamin A and 13,900 IU Vitamin D kg^{-1} DM. Wheat middlings were used as the carrier.

Scientific, Edmonton, AB). Concentrations of phosphorus (P), calcium (Ca), magnesium (Mg), copper (Cu) and zinc (Zn) were determined by first dry ashing a 1 g sample at 550°C for 12 hours. Sample content of P was determined colormetrically (AOAC; 7.103-7.105; 1975) using a Bausch and Lomb Spectronic 20 (Fisher Scientific, Edmonton, AB). Calcium, Mg, Cu and Zn content were determined (AOAC; 7.077-7.082; 1975) using flame atomic absorption (Instrument Laboratory AA/AE Spectrophotometric Model 551, Instrument Laboratory Inc., Wilmington, Massachusetts). Total sulfur (S) content was determined according to Boila et al. (1984). Gramine and hordenine concentrations in forage samples were determined by gas-liquid chromatography (Duynisveld et al. 1990) from the frozen subsample of each composite.

Statistical Analysis

Forage composition was analyzed as a completely randomized design with paddocks nested within cultivar as main effect and sampling date as sub effect. Composition of forage was analyzed as two separate periods using analysis of variance (SAS Institue Inc. 1985). There were seven sampling dates for CP, ADF, NDF, gramine and hordenine concentrations and three sampling dates for Ca, P, Mg, S, Cu and Zn content in each period. Differences between cultivars were tested with the pen within cultivar error term. Differences between sampling dates were tested using the sampling date and pen within cultivar error term. Mean separations were performed for cultivars using Duncan's multiple-range test using the pen within cultivar effects as the error term.

Regression equations for hordenine, gramine and CP were generated with Nonlinear Procedures (SAS 1985) using the weekly determinations of each factor.

Weight gains of lambs during the two periods of the grazing trial were analyzed as a split plot design with cultivar as the main effects and period as a sub effect. Paddocks were nested within cultivars. The trial was analyzed using general linear models (SAS 1985). Differences between cultivars were tested with the pen within cultivar error term. Period and the interaction of period and cultivar were tested with the interaction of period and pen within cultivar error term.

Weight gains of lambs on the finishing ration were analyzed as lambs nested within cultivars using general linear models (SAS 1985). Differences between cultivars were tested with lamb within cultivar as the error term. Mean separations were performed with Bonferroni's Inequalities Test.

RESULTS AND DISCUSSION

Forage

There were no differences (P>0.05) in hordenine concentration between cultivars for either period I or period II (Table 13 and 14, respectively), although a larger range in values was observed for period I relative to period II (Figure 2). Generally, hordenine concentrations were increasing during period I and decreasing during period II.

Forage gramine concentrations were not different (P>0.05) between cultivars for period I, however, Rival concentrations were higher (P<0.05) than Venture and Frontier in period II (Table 13 and 14, respectively). Gramine concentrations were more variable in period I (Figure 3) than in period II. Forage gramine concentrations were low initially and increased dramatically with five of six paddocks achieving peak concentrations during period I. A gradual decline was observed for all paddocks during period II.

The concentration of gramine for the RCG cultivars in this experiment are within the range of the total indole alkaloid concentrations reported in other animal performance trials in the literature, with values of 0.8 mg g^{-1} to 4.0 mg g^{-1} total indole alkaloid (Marten et al. 1976, 1981). However, the trials of Marten include cultivars which were classed as high alkaloid whereas Rival and Venture are classed as low alkaloid. This elevation of alkaloid level may be attributed to the continuous grazing and drought conditions experienced during 1988.

Using the suggested value of 2 mg g^{-1} indole alkaloid as a threshold concentration above which lamb gains are reduced (Marten et al. 1981), lambs grazing the two pens of Rival were exposed to levels of

TABLE 13. Nutrient and alkaloid concentration, DM basis, of reed canarygrass cultivars during period I (May 24 to July 5, 1988) of the grazing trial.

	Frontier	Rival	Venture	Standard Error
Crude protein (%)†	19.9	20.3	18.8	0.7
Acid detergent fiber (%)†	32.6	31.5	33.4	0.9
Neutral detergent fiber (%)†	61.9	62.0	63.9	0.6
Calcium (g kg^{-1})‡	2.7	3.2	2.7	0.3
Phosphorus (g kg^{-1})‡	4.0	3.7	4.0	0.2
Magnesium (g kg^{-1})‡	3.2	3.4	3.2	0.1
Sulfur (g kg^{-1})‡	3.3	2.8	3.0	0.2
Copper (mg kg^{-1})‡	7.0	6.7	7.1	0.1
Zinc (mg kg^{-1})‡	31.7	26.2	27.6	1.7
Hordenine (mg g^{-1})†	2.76	3.46	2.51	0.34
Gramine (mg g^{-1})†	1.93	3.21	2.00	0.62

† means of seven sampling dates for each of two paddocks.
‡ means of three sampling dates for each of two paddocks.

TABLE 14. Nutrient and alkaloid concentration, DM basis, of reed canarygrass cultivars during period II (July 19 to August 30, 1988) of the grazing trial.

	Frontier	Rival	Venture	Standard Error
Crude protein (%)†	16.0	15.1	14.7	0.4
Acid detergent fiber (%)†	34.2b	33.7b	35.8a	0.3
Neutral detergent fiber (%)†	62.0b	62.6b	64.6a	0.3
Calcium (g kg^{-1})‡	3.5	3.8	3.8	0.3
Phosphorus (g kg^{-1})‡	2.8	2.3	2.8	0.2
Magnesium (g kg^{-1})‡	3.8	3.9	3.7	0.2
Sulfur (g kg^{-1})‡	3.3	3.2	3.0	0.3
Copper (mg kg^{-1})‡	5.1	4.3	4.4	0.5
Zinc (mg kg^{-1})‡	20.6	14.7	21.7	1.8
Hordenine (mg g^{-1})†	2.72	3.07	2.10	0.17
Gramine (mg g^{-1})†	2.42b	3.75a	2.27b	0.08

a-b means in rows with different letters are different ($P<0.05$).
† means of seven sampling dates for each of two paddocks.
‡ means of three sampling dates for each of two paddocks.

FIGURE 2: Hordenine concentration (mg g^{-1} DM) of three cultivars of reed canarygrass during summer grazing 1988. Concentrations estimated with the use of nonlinear regression equations.

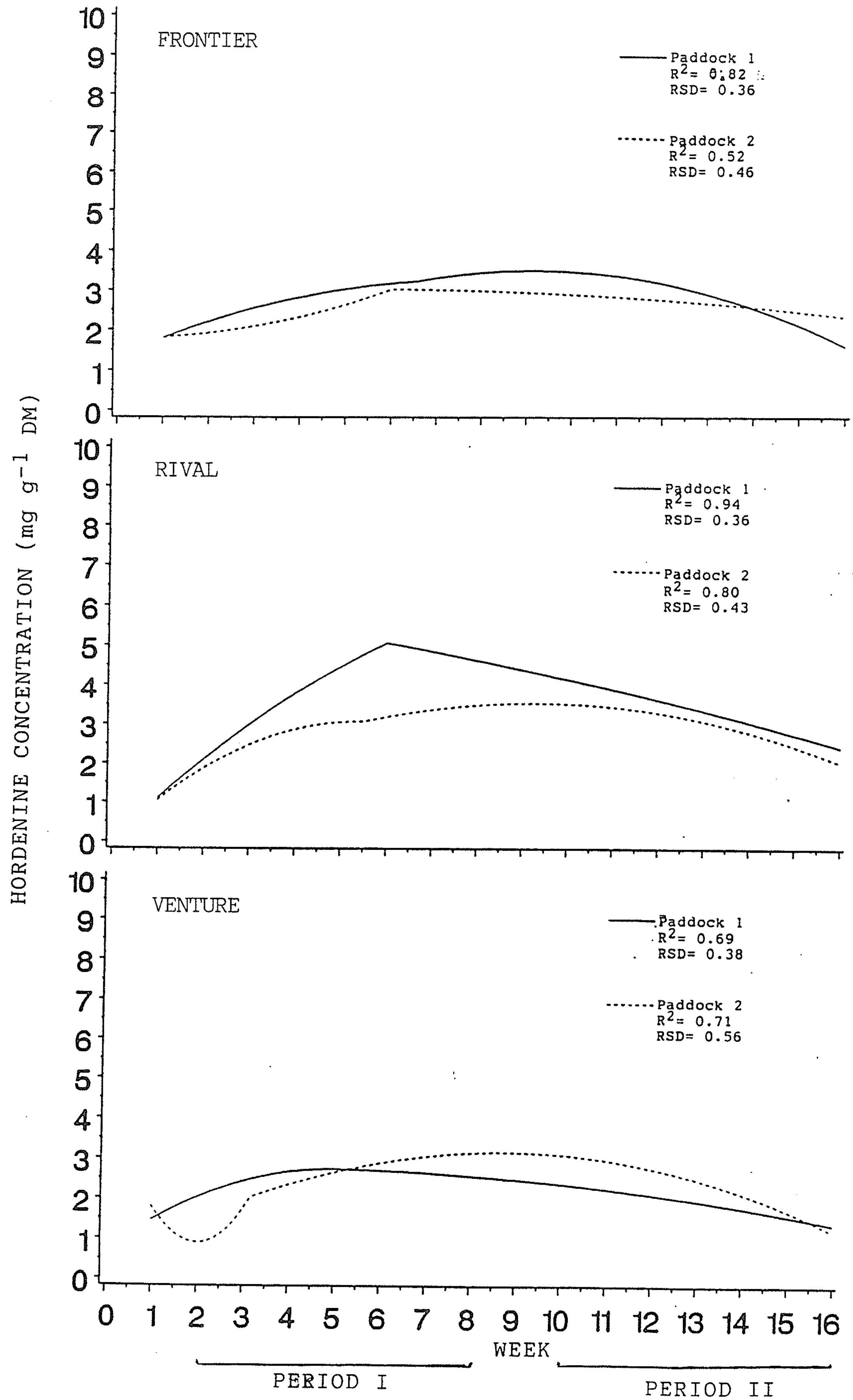
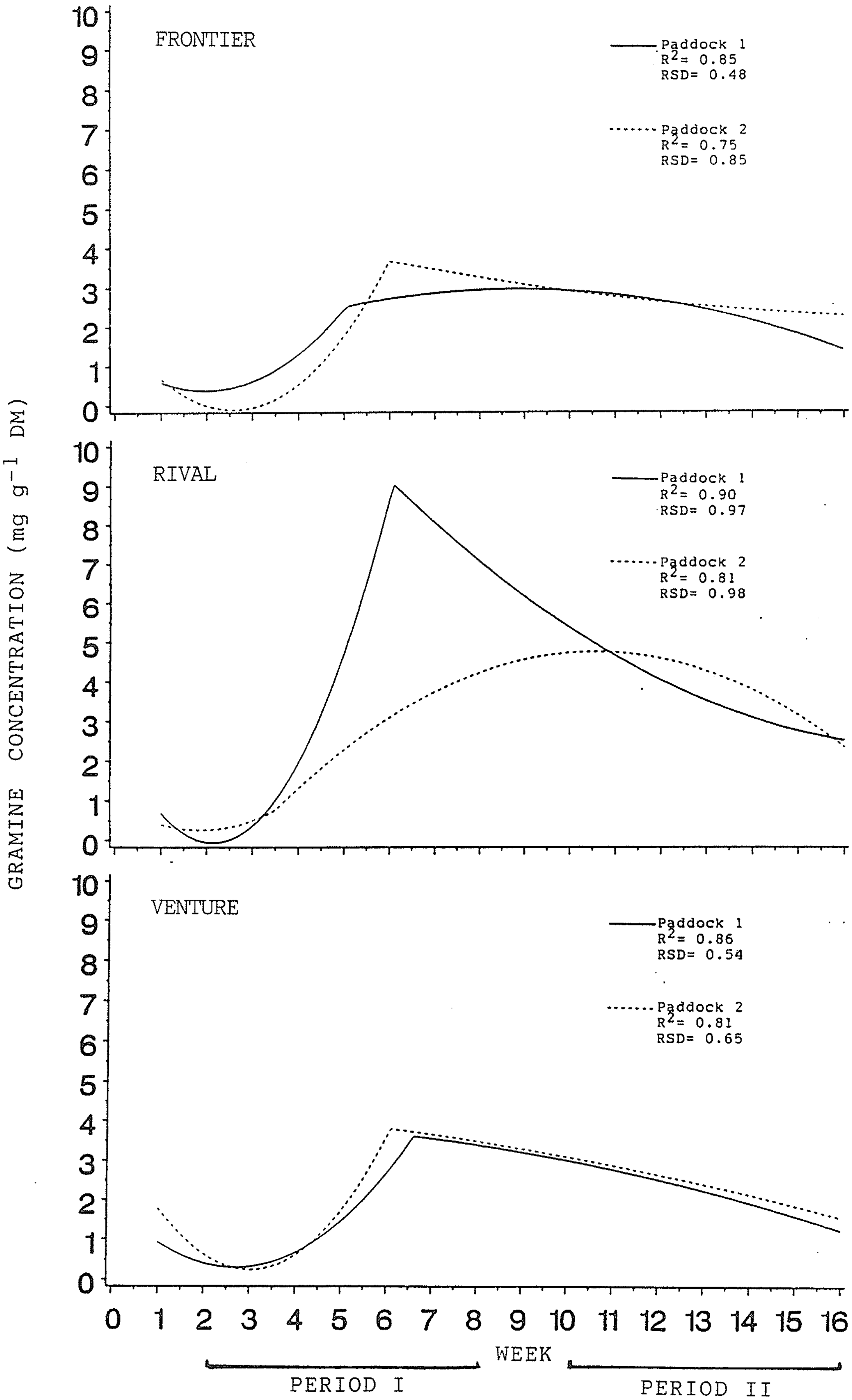

FRONTIER
Paddock 1
R2= 0.82
RSD= 0.36
Paddock 2
R2= 0.52
RSD= 0.46
RIVAL
Paddock 1
R2= 0.94
RSD= 0.36
Paddock 2
R2= 0.80
RSD= 0.43
VENTURE
Paddock 1
R2= 0.69
RSD= 0.38
Paddock 2
R2= 0.71
RSD= 0.56
HORDENINE CONCENTRATION (mg g-1 DM)
0 1 2 3 4 5 6 7 8 9 10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEK
PERIOD I
PERIOD II

FIGURE 3: Gramine concentration (mg g^{-1} DM) of three cultivars of reed canarygrass during summer grazing 1988. Concentrations estimated with the use of nonlinear regression equations.



GRAMINE CONCENTRATION (mg g-1 DM)
FRONTIER
Paddock 1
R2= 0.85
RSD= 0.48
Paddock 2
R2= 0.75
RSD= 0.85
RIVAL
Paddock 1
R2= 0.90
RSD= 0.97
Paddock 2
R2= 0.81
RSD= 0.98
VENTURE
Paddock 1
R2= 0.86
RSD= 0.54
Paddock 2
R2= 0.81
RSD= 0.65
0 1 2 3 4 5 6 7 8 9 10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEK
PERIOD I
PERIOD II

gramine capable of reducing gains for 24.9 ± 2.1 days in period I and 42.0 ± 1.8 days in period II (Figure 2). Lambs grazing Venture were exposed to these levels for the shortest period of time, 18.6 ± 2.1 and 35.9 ± 1.8 days in period I and II, respectively, whereas lambs grazing Frontier were exposed for an intermediate period of time, 21.7 ± 2.1 and 40.0 ± 1.8 days in period I and II, respectively.

Animal Performance: Grazing Trial

There were differences (P=0.07) among cultivars for animal weight gain. Lambs grazing Frontier, Rival and Venture had average daily gains (ADG) of 119, 146 and 161 ± 8 g, respectively. ADG (mean ± SE) for lambs in period I (153 ± 6 g) were greater (P=0.09) than period II (131 ± 6 g). An interaction (P=0.06) between cultivar and period was observed for lamb ADG.

Lambs grazing Frontier tended to have lower ADG than lambs grazing Venture during period I (Table 15). This did not appear to be related to forage nutrient content as there were no differences (P>0.05) in the nutrient profiles of the three RCG cultivars in period I (Table 13). The observed incidence of diarrhea was greater (P<0.05) in the Frontier paddocks than in the Rival or Venture paddocks with 54.6, 4.6 and 4.6 ± 6.4 % of lambs exhibiting symptoms, respectively. Preferential grazing of bluegrass (*Poa* spp.) and quackgrass (*Elymus repens*) was observed in the Frontier paddocks but not in the Rival or Venture paddocks. Diarrhea in lambs grazing RCG is an indication of the tryptamine and β-carboline type alkaloids present in Frontier (Hovin and Marten 1975). These alkaloids could be responsible for the reduced lamb gains and selective grazing of Frontier because Rival and Venture are free of the tryptamine and β-carboline aklaloids.

TABLE 15. Weight gain for lambs assigned to three cultivars of reed canarygrass during period I (May 24 to July 5, 1988) and period II (July 19 to August 30, 1988) of grazing trial.

	Number of Lambs	Initial Weight (kg)	Average Daily Gain (g)
Period I			
Frontier	19	17.4 ± 0.5†	108 ± 11
Rival	18	18.5 ± 0.6	158 ± 11
Venture	20	18.0 ± 0.6	193 ± 10
Period II			
Frontier	19	21.8 ± 1.2	130 ± 11
Rival	18	25.2 ± 1.2	134 ± 11
Venture	20	26.8 ± 1.2	128 ± 10
Significance			
Cultivar			0.07
Period			0.09
Cultivar*Period			0.06

† Least square means ± standard error.

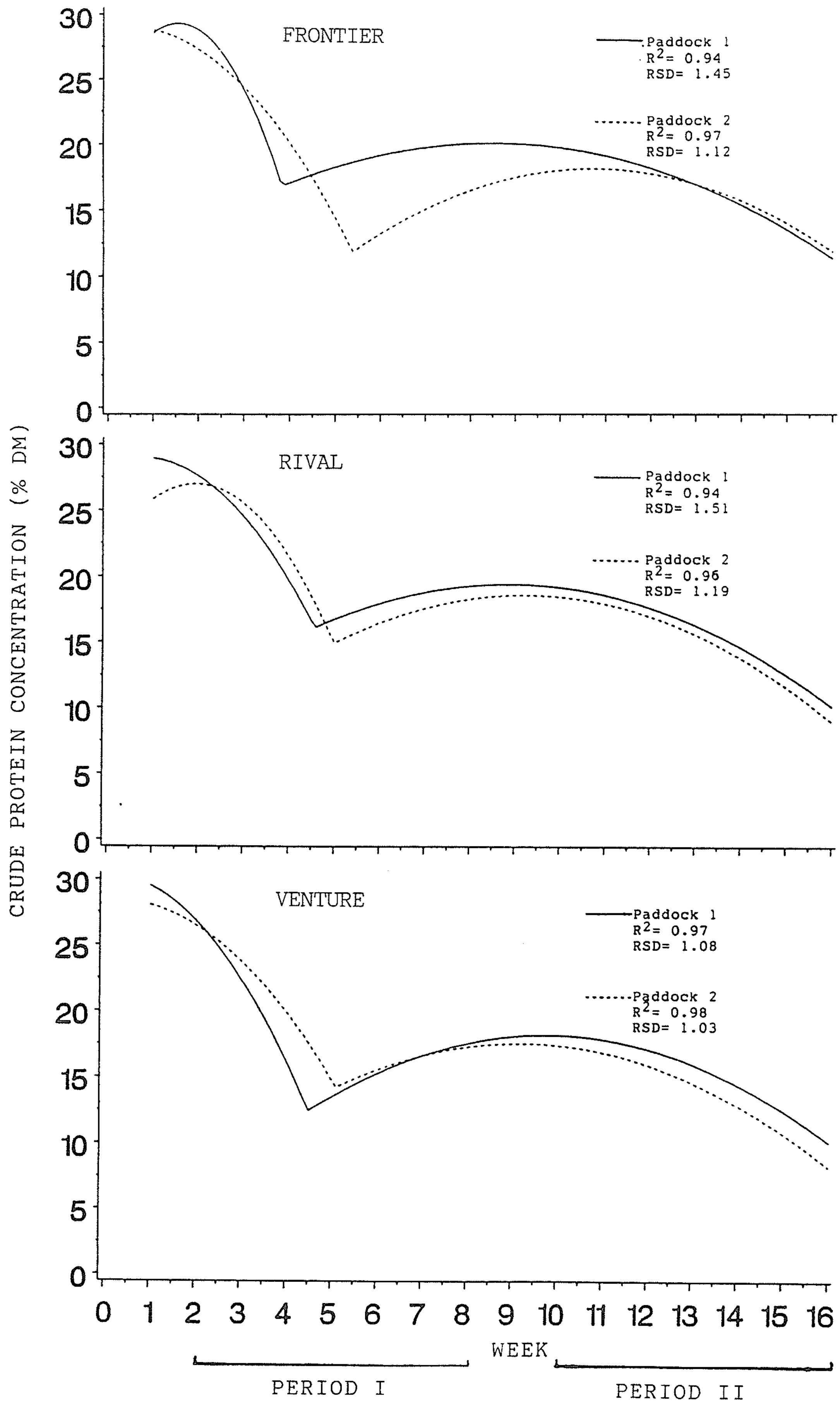
The weight gains of lambs grazing Rival tended to be intermediate to Venture and Frontier (Table 15). A longer period of exposure to gramine levels above 2 mg g^{-1} DM (Marten et al. 1981) could explain the trend of reduced gains for lambs grazing Rival as compared to Venture in period I (Figure 3).

In period II, there were no apparent differences in lamb ADG among cultivars (Table 15). Exposure of lambs to dietary levels of gramine above 2 mg g^{-1} (Figure 3) over an extended period of time may have resulted in gains which were lower than expected for lambs grazing Rival and Venture paddocks. The incidence of diarrhea was still higher ($P<0.05$) in the Frontier paddocks (72.7, 4.6 and 0.0 ± 5.9 % for Frontier, Rival and Venture, respectively), but preferential grazing against RCG was observed for lambs grazing in all three cultivars. There were no differences ($P>0.05$) in nutrient composition in period II with the exception of the higher ($P<0.05$) ADF and NDF content for Venture than for Rival and Frontier (Table 14). Higher fiber values also may have reduced the of ADG for lambs grazing Venture in period II as compared to period I.

In addition, CP levels may have been limiting in the later portions of period I and II (Figure 4), contributing to the depression ADG of lambs in period II (Table 15). At the time when gramine levels were decreasing below 2 mg g^{-1} in period II (Figure 3), CP levels may have been limiting.

One lamb was removed from a paddock containing Rival after demonstrating syptoms of sunstroke. Also, one lamb from each of a Rival and Venture paddock was removed due to poor weight gains associated with a previous exposure to coccidiosis. Weight gain data from these three

FIGURE 4: Crude protein concentration (mg g^{-1} DM) of three cultivars of reed canarygrass during summer grazing 1988. Concentrations estimated with the use of nonlinear regression equations.



FRONTIER
Paddock 1
R2= 0.94
RSD= 1.45
Paddock 2
R2= 0.97
RSD= 1.12
RIVAL
Paddock 1
R2= 0.94
RSD= 1.51
Paddock 2
R2= 0.96
RSD= 1.19
VENTURE
Paddock 1
R2= 0.97
RSD= 1.08
Paddock 2
R2= 0.98
RSD= 1.03
CRUDE PROTEIN CONCENTRATION (% DM)
WEEK
PERIOD I
PERIOD II

lambs was not included in the analysis.

Even though lambs grazing RCG cultivars had reduced ADG associated with the elevated alkaloid concentrations, lamb growth was supported under drought conditions. The paddocks containing timothy did not provide sufficient forage to sustain lamb growth from the fifth week in period I until the end of the summer. The animal unit grazing days paddock^{-1} for timothy were 47.6 in period I and 0 in period II whereas Venture, Frontier and Rival provided 89.0, 85.5 and 74.2 ± 8.1, and 32.3, 32.1 and 31.7 animal unit grazing days, respectively, for period I and II.

Animal Performance: Finishing Trial

The finishing ration was formulated to meet or exceed nutrient requirements for growing lambs (NRC 1985, Table 12). Data for one animal assigned to Venture was not included in the analysis because of poor performance throughout the finishing trial. Forage consumed during the grazing season did not influence ($P>0.05$) average daily gains of lambs consuming finishing rations during the test period under feedlot conditions (Table 16). It would appear that there were no residual affects of the alkaloids on lambs removed from pasture because none of the differences among cultivars in alkaloid type and content in the RCG forage were reflected in lamb gains during the finishing trial.

TABLE 16. Weight gain for lambs assigned to each cultivar of grass while on finishing ration (Sept. 12 to Oct. 24, 1988).

	Reed Canarygrass			Timothy
	Frontier	Rival	Venture	Champ
Number of Lambs	7	7	6	7
Initial weight (kg)†	28.3 ± 1.8	29.9 ± 1.8	29.5 ± 1.9	32.8 ± 1.8
Final weight (kg)†	45.5 ± 2.0	47.9 ± 2.0	47.1 ± 2.1	49.8 ± 2.0
Weight gain (kg)†	17.2 ± 1.0	18.0 ± 1.0	17.6 ± 1.1	17.1 ± 1.0
Average daily gain (g)†	307 ± 18	321 ± 18	314 ± 19	305 ± 18

† Least square means ± standard error.

CONCLUSIONS

Cultivar of RCG influenced (P=0.07) ADG of lambs during the grazing season. Weight gains of lambs were reduced (P=0.09) in period II as compared to period I and an interaction (P=0.06) was observed between cultivar and period. In period I, lambs grazing Frontier tended to have reduced lamb ADG and increased incidence of diarrhea as compared to Venture, even though gramine and hordenine concentrations in the cultivars were not different (P>0.05). The reduced ADG and diarrhea maybe the result of the tryptamines and β-carbolines, which have been detected in Frontier. Lambs grazing Rival had intermediate ADG, possibly due to longer period of exposure to increased gramine concentrations. In period II, no apparent differences were detected between cultivars of RCG for ADG of grazing lambs, even though there were differences (P<0.05) between cultivars for gramine and fiber concentrations. It appears that the weight gains of lambs from each of Rival and Venture were reduced because of a higher (P<0.05) gramine concentration for Rival and higher (P<0.05) ADF and NDF content in combination with high gramine levels for the Venture. No residual effects (P>0.05) of the alkaloids were detected to influence lamb average daily gains when removed from pastures and place on a commerical finishing ration.

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GENERAL DISCUSSION

Intake of RCG and Timothy Forage

Differences ($P>0.05$) in DM intake were not detected between RCG and timothy in initial growth or in regrowth harvested in 1987, when grasses were harvested at similar stages of maturity. Differences ($P>0.05$) in DM intake were not detected between RCG cultivars. Intake values for RCG cultivars tended to be lower in regrowth than for initial growth. With the increase in gramine and hordenine concentration in regrowth, the initial suggestion was that there may be an effect of the alkaloids on intake. However, Rival had the highest gramine and hordenine concentration and also the highest intake for RCG regrowth, therefore, it is suggested that alkaloid type or concentration did not appear to influence DM intake at concentrations detected in the forage.

Digestibility of RCG and Timothy Forage

Initial growth and regrowth of RCG harvested in 1987 had lower ($P<0.05$) DM and fiber digestibilities than timothy when ADF values were similiar. There were differences ($P<0.05$) between RCG cultivars with Rival having higher DM and fiber digestibilities than Frontier and Venture. Rival had the highest gramine and hordenine concentrations, therefore, it is suggested that the alkaloid types and concentrations at the levels detected did not influence digestibilities of DM and fiber components.

Grazing Trial of RCG Forage

In the 1988 grazing trial, differences ($P=0.07$) in ADG of lambs were detected among cultivars of RCG; means (± SE) were 119, 146 and 161 ± 6 g for Frontier, Rival and Venture, respectively. An interaction ($P=0.06$) between cultivar and period was detected.

Venture paddocks tended to have higher ($P>0.05$) ADG than Frontier paddocks in period I. There were no differences ($P<0.05$) in forage nutrient or alkaloid concentration among the three cultivars for that period. Lambs grazing Frontier exhibited a higher ($P<0.05$) incidence of diarrhea than lambs grazing Rival or Venture (54.6, 4.6 and 4.6 ± 6.4, respectively) and preferential grazing of other grass species was observed in Frontier paddocks but not in Rival or Venture paddocks. Increased incidence of diarrhea for lambs grazing RCG maybe attributed to the presence of tryptamines and β-carbolines, which have been detected Frontier (Marten and Hovin 1975). Lambs grazing Rival tended to have intermediate ADG to Frontier and Venture. The number of days in period I when gramine was above 2 mg g^{-1} DM was higher for Rival than Venture (24.9 and 18.6 ± 2.1 days, respectively) and this may have reduced the ADG of lambs grazing Rival.

In period II of the 1988 grazing trial, no apparent differences were detected among cultivars for ADG of grazing lambs. Preferential grazing was exhibited against all cultivars. The lack of separation between cultivars for lamb ADG is difficult to explain. Rival forage had a higher ($P<0.05$) gramine concentration than Venture or Frontier. Venture forage may have been limited because of higher ($P<0.05$) ADF and NDF contents. These factors may have reduced the ADG of lambs grazing the two cultivars to levels observed for lambs grazing on Frontier paddocks, where lambs were still exhibiting diarrhea.

Even though the ADG of lambs grazing the RCG cultivars were reduced, the RCG paddocks could provide enough forage for lambs to grow. Because of the dry conditions in 1988, the timothy paddocks could not support lamb growth from the second last week of period I to the end of the trial.

There were no differences ($P>0.05$) between cultivars for weight gains of lambs when lambs were removed from RCG paddocks and placed on a commercial finishing ration. Lambs which had been grazing alkaloid free material were placed with the RCG lambs on the finishing ration and no differences ($P>0.05$) were detected. Therefore, it seems that there was no detected residual affect of gramine and hordenine on animal performance.

Gramine in RCG Forage

Gramine concentration in RCG forage is variable and influenced by the stage of growth, conditions under which the grass is grown and genotype. Gramine concentration for initial growth was lower ($P<0.05$) than regrowth (0.28 ± 0.04 and 1.19 ± 0.05 mg g^{-1} DM, respectively) for five cultivars at two off campus sites in 1988. Initial growth of RCG in the grazing trial at the campus site in 1987 had gramine concentrations below 0.5 mg g^{-1} DM and later in the grazing season, regrowth surpassed concentrations of 4 mg g^{-1} DM. In 1988, the campus site was experiencing drought (148 mm precipitation from May to July) and these conditions were thought to raise gramine concentration. This is in contrast to 1987 (292 mm precipitation from May to July) when gramine concentrations were not detected in initial growth and regrowth had gramine concentrations ranging from 0.52 to 1.23 mg g^{-1} DM.

In 1988 at the campus site, the RCG forage in grazing trials was continuously grazed, which would tend to increase gramine concentration as compared to 1987 when the material was cut at heading for initial growth and six leaf vegetative for regrowth. Genotype or cultivar influenced gramine concentration with Rival tending to or having a higher ($P<0.05$) concentration than Venture. The cultivars of Frontier, Castor, Vantage and Palaton had intermediate gramine concentrations.

When conducting grazing trials with RCG, it is important to account for variability by sampling on a frequent basis to depict changes in concentration over time. In period I of the 1988 grazing trial at the campus site, gramine concentration increased dramatically, peaked, and then declined. In period II, the gramine concentrations were decreasing but at a much slower rate than with which they had increased during period I.

The technique developed for quantifying gramine allows for determination of gramine concentration in the presence or absence of tryptamines and β-carbolines. For the cultivars tested, it was determined that the gramine concentrations in the recently released tryptamine and β-carboline free cultivars are in the same range as concentrations of the older tryptamine and β-carboline cultivars. These new cultivars were selected for low gramine concentration from tryptamine and β-carboine free material but it seems the levels are not necessarily lower than gramine concentrations in the older cultivars.

Hordenine in RCG Forage

Hordenine concentration in RCG forage tended to be less variable than gramine concentration. Hordenine values for initial growth at the campus site in 1987 ranged from 0.53 to 0.68 mg g^{-1} DM and regrowth values were 1.31 to 1.41 mg g^{-1} DM. In 1988 at the two off campus sites, average hordenine concentration for the five cultivars was not different ($P>0.05$) between initial and regrowth (1.84 and 1.86, respectively). In 1988, at the campus site, period I of the grazing trial tended to have a higher concentration than period II. Hordenine concentrations for Rival, Frontier and Venture were 3.46, 2.76 and 2.51 ± 0.34 mg g^{-1} DM, respectively, for period I, and 3.07, 2.72 and 2.10 ± 0.17 mg g^{-1} DM, respectively, for period II. The dry year of 1988 seemed to increase hordenine concentration as compared to 1987 at the campus site.

No differences (P>0.05) between RCG cultivars for hordenine concentration were detected. Hordenine concentrations in cultivars either containing or free of tryptamine and β-carboline alkaloids seem to be similiar. Increases or decreases in hordenine concentration were paralleled by increases or decreases in gramine concentration but on a smaller scale. The quantification technique developed provided a higher recovery of both hordenine and gramine without the use of separate extraction procedures.

Forage quality

For the 1988 grazing trial, determinations of quality components in forage, such as gramine, hordenine, CP, ADF and NDF, were based on samples collected on a weekly basis during the grazing season. These determinations (16 dates) were used to calculate nonlinear regression equations for each component in each paddock. Results for gramine and hordenine were previously discussed. Forage CP values were initially high in period I, and decreased until the final 7-10 days after which some improvement in CP levels was observed. In period II, CP content decreased gradually. Both ADF and NDF contents (Appendix 22 and 23) in period I increased to a peak concentration and then decreased. In period II, there was a gradual increase in forage fiber contents. Variability from the estimated concentrations of each component increased from CP to ADF to NDF suggesting the potential inconsistency with using CP as a sole predicter of forage quality.

Even with the use of weekly determinations and regressional analysis of each nutritional component, it is difficult to partition what portion of the change is related to changes in the plant itself and what portion is the result of DM removal by the lambs. However, as compared to a period mean, regressional analysis of each component does provide a more complete profile of the forage available to the grazing animal while that animal is being tested.

The method of feeding fresh frozen forage used for the digestibility and intake trials was chosen because it preserved the alkaloids. In addition, this method removes the variability due change in composition while the evaluation of the forage is preformed using lambs. However, it only represents one point during the grazing season for that forage and the animal's ability to select plant parts is removed.

CONCLUSIONS

1. Consistent differences in nutrient composition were not observed among the three cultivars of reed canarygrass (RCG) when evaluated at the same stage of growth. The cultivars of RCG tended to have higher crude protein contents and reduced digestibilities as compared to Champ timothy.

2. Pasture productivity was influenced by the species of grass under drought conditions; timothy could not support animal growth whereas the RCG cultivars could. Within the three RCG cultivars, gains of lambs grazing Rival and Venture were better than or equal to the gains of lambs grazing Frontier.

3. Forage of Rival and Venture is an improvement over Frontier because of the absence of the tryptamine and β-carboline alkaloids. However, concentrations of gramine and hordenine which reduced lamb weight gain were observed in these two cultivars.

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APPENDICES

APPENDIX 1: Individual data on hordenine and gramine concentration (mg g^{-1}) determined using gas-liquid chromatography comparing cultivar, stage of growth and location. Study I.

VARIETY	LOC	REP	CUT	HORDENIN	GRAMINE
CASTOR	A	1	1	2.11074	0.37979
CASTOR	A	2	1	2.75769	0.45440
CASTOR	A	1	2	1.73359	1.06501
CASTOR	A	2	2	2.23683	1.12663
CASTOR	B	1	1	1.06644	0.33326
CASTOR	B	2	1	1.45505	0.15322
CASTOR	B	1	2	2.32391	1.27779
CASTOR	B	2	2	.	.
PALATON	A	1	1	2.39752	0.22850
PALATON	A	2	1	2.14301	0.34953
PALATON	A	1	2	1.53464	1.04757
PALATON	A	2	2	1.72211	1.72624
PALATON	B	1	1	1.21835	0.22614
PALATON	B	2	1	1.79448	0.29980
PALATON	B	1	2	1.37652	0.67479
PALATON	B	2	2	1.96774	1.01353
RIVAL	A	1	1	2.24788	0.25239
RIVAL	A	2	1	2.33280	0.40043
RIVAL	A	1	2	1.34203	0.85224
RIVAL	A	2	2	2.05949	1.49444
RIVAL	B	1	1	2.16482	0.51559
RIVAL	B	2	1	1.49288	0.36364
RIVAL	B	1	2	1.77757	1.53902
RIVAL	B	2	2	2.80586	2.05748
VANTAGE	A	1	1	2.27915	0.28368
VANTAGE	A	2	1	2.58652	0.46609
VANTAGE	A	1	2	1.34459	0.94513
VANTAGE	A	2	2	1.95092	1.09610
VANTAGE	B	1	1	1.45786	0.16855
VANTAGE	B	2	1	1.13646	0.14179
VANTAGE	B	1	2	2.06483	1.46407
VANTAGE	B	2	2	1.81951	1.20503
VENTURE	A	1	1	1.54768	0.14040
VENTURE	A	2	1	1.74690	0.18721
VENTURE	A	1	2	1.44397	0.78612
VENTURE	A	2	2	1.32613	0.78498
VENTURE	B	1	1	1.20438	0.07729
VENTURE	B	2	1	1.67387	0.29427
VENTURE	B	1	2	1.54757	1.12112
VENTURE	B	2	2	1.78071	1.28060

VARIETY=cultivar LOC=location REP=replicate CUT=stage of growth
1=initial growth 2=regrowth

APPENDIX 2: Analysis of variance for alkaloid concentration in RCG forage. Study I.

Parameter	Source	df	Type III SS	F value	Pr > F
Gramine	Loc	1	0.0435	0.36	0.6080
	rep(loc)	2	0.2396	3.98	0.0708
	cultivar	4	0.5164	4.50	0.0337
	loc*cultivar	4	0.4811	4.20	0.0403
	cultivar*rep(loc)	8	0.2294	0.95	0.5350
	cut	1	7.2811	202.02	0.0049
	loc*cut	1	0.1430	3.97	0.1846
	cut*rep(loc)	2	0.0701	1.19	0.3587
	cut*cultivar	4	0.1052	0.87	0.5270
	loc*cut*cultivar	4	0.3815	3.15	0.0884
	Error	7	0.2118		
Hordenine	Loc	1	0.2903	0.79	0.4677
	rep(loc)	2	0.7343	3.44	0.0911
	cultivar	4	1.3983	3.74	0.0533
	loc*cultivar	4	0.3955	1.06	0.4368
	cultivar*rep(loc)	8	0.7487	0.88	0.5753
	cut	1	0.0044	0.05	0.8505
	loc*cut	1	2.8660	29.73	0.0320
	cut*rep(loc)	2	0.1928	0.90	0.4477
	cut*cultivar	4	0.4385	1.03	0.4557
	loc*cut*cultivar	4	0.4816	1.13	0.4156
	Error	7	0.7469		

LOC=location REP=replicate CUT=stage of growth

APPENDIX 3: Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
C	2	M	1	0.588798	2.29104
C	2	M	2	0.585433	2.27795
C	2	M	3	0.544663	2.11931
C	2	M	4	0.563844	2.19395
C	2	M	5	0.594710	2.31405
C	2	M	6	0.551212	2.14480
C	2	M	7	0.574541	2.23557
C	8	M	1	0.261714	1.09275
C	8	M	2	0.195697	0.81711
C	8	M	3	0.390206	1.62925
C	8	M	4	0.540373	2.25625
C	8	M	5	0.643608	2.68730
C	8	M	6	0.628727	2.62517
C	8	M	7	0.659400	2.75324
C	12	M	1	0.747722	2.94960
C	12	M	2	0.751151	2.96312
C	12	M	3	0.693108	2.73415
C	12	M	4	0.738920	2.91487
C	12	M	5	0.806069	3.17976
C	12	M	6	0.732006	2.88760
C	12	M	7	0.759871	2.99752
C	15	M	1	0.623153	2.43896
C	15	M	2	0.689432	2.69836
C	15	M	3	0.642058	2.51295
C	15	M	4	0.682685	2.67196
C	15	M	5	0.729422	2.85488
C	15	M	6	0.641278	2.50990
C	15	M	7	0.659154	2.57986
C	17	F	1	0.614671	2.89257
C	17	F	2	0.710911	3.34546
C	17	F	3	0.606774	2.85541
C	17	F	4	0.602549	2.83552
C	17	F	5	0.636952	2.99742
C	17	F	6	0.599583	2.82157
C	17	F	7	0.634929	2.98790
C	23	F	1	0.455695	2.04807
C	23	F	2	0.385016	1.73041
C	23	F	3	0.297297	1.33617
C	23	F	4	0.472771	2.12482
C	23	F	5	0.427631	1.92194
C	23	F	6	0.468160	2.10409
C	23	F	7	0.530703	2.38518
C	27	F	1	0.807841	3.28390
C	27	F	2	0.904100	3.67520
C	27	F	3	0.789705	3.21018
C	27	F	4	0.709770	2.88524
C	27	F	5	0.810708	3.29556
C	27	F	6	0.750468	3.05068
C	27	F	7	0.812378	3.30235
C	32	F	1	0.678444	2.51742
C	32	F	2	0.691032	2.56413
C	32	F	3	0.752569	2.79246
C	32	F	4	0.721149	2.67588
C	32	F	5	0.602816	2.23679
C	32	F	6	0.675918	2.50804

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 3 (cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
C	32	F	7	0.65422	2.42753
C	35	F	1	0.72288	3.00576
C	35	F	2	0.70931	2.94933
C	35	F	3	0.65801	2.73601
C	35	F	4	0.62236	2.58777
C	35	F	5	0.68857	2.86307
C	35	F	6	0.62946	2.61731
C	35	F	7	0.65474	2.72241
C	39	F	1	0.95378	3.02309
C	39	F	2	1.04389	3.30869
C	39	F	3	0.80909	2.56446
C	39	F	4	0.97837	3.10101
C	39	F	5	1.05170	3.33344
C	39	F	6	0.86105	2.72916
C	39	F	7	0.92816	2.94186
F	1	M	1	0.62793	2.52181
F	1	M	2	0.60049	2.41160
F	1	M	3	0.60470	2.42851
F	1	M	4	0.66728	2.67983
F	1	M	5	0.66255	2.66084
F	1	M	6	0.66814	2.68328
F	1	M	7	0.74436	2.98938
F	6	M	1	0.69795	2.53338
F	6	M	2	0.63706	2.31239
F	6	M	3	0.64859	2.35421
F	6	M	4	0.70127	2.54543
F	6	M	5	0.71094	2.58054
F	6	M	6	0.68286	2.47864
F	6	M	7	0.71932	2.61095
F	11	M	1	0.51711	2.41639
F	11	M	2	0.48310	2.25746
F	11	M	3	0.42294	1.97634
F	11	M	4	0.50024	2.33758
F	11	M	5	0.53613	2.50529
F	11	M	6	0.55763	2.60574
F	11	M	7	0.68760	3.21308
F	16	M	1	0.66401	2.37145
F	16	M	2	0.71166	2.54163
F	16	M	3	0.70009	2.50034
F	16	M	4	0.70617	2.52202
F	16	M	5	0.72063	2.57366
F	16	M	6	0.72645	2.59448
F	16	M	7	0.82123	2.93295
F	18	F	1	0.59166	2.66512
F	18	F	2	0.52548	2.36704
F	18	F	3	0.52773	2.37715
F	18	F	4	0.55765	2.51194
F	18	F	5	0.57758	2.60170
F	18	F	6	0.57287	2.58048
F	18	F	7	0.59046	2.65974
F	24	F	1	0.77637	2.94078
F	24	F	2	0.76523	2.89860
F	24	F	3	0.72343	2.74026
F	24	F	4	0.68568	2.59727
F	24	F	5	0.63107	2.39042

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 3 (cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
F	24	F	6	0.686251	2.59943
F	24	F	7	0.894203	3.38713
F	26	F	1	0.878482	3.25364
F	26	F	2	0.869957	3.22206
F	26	F	3	0.825949	3.05907
F	26	F	4	0.822943	3.04794
F	26	F	5	0.839576	3.10954
F	26	F	6	0.820170	3.03767
F	26	F	7	0.892402	3.30519
F	29	F	1	0.362409	1.85375
F	29	F	2	0.298522	1.52697
F	29	F	3	0.209640	1.07233
F	29	F	4	0.347467	1.77732
F	29	F	5	0.364636	1.86515
F	29	F	6	0.376284	1.92473
F	29	F	7	0.516873	2.64385
F	34	F	1	0.641462	2.60228
F	34	F	2	0.674556	2.73654
F	34	F	3	0.676836	2.74578
F	34	F	4	0.745503	3.02435
F	34	F	5	0.742889	3.01375
F	34	F	6	0.739543	3.00017
F	34	F	7	0.829548	3.36531
F	40	F	1	0.787080	3.15463
F	40	F	2	0.886185	3.55185
F	40	F	3	0.797386	3.19594
F	40	F	4	0.730225	2.92675
F	40	F	5	0.735539	2.94805
F	40	F	6	0.720945	2.88956
F	40	F	7	0.767993	3.07813
R	4	M	1	0.823007	2.62104
R	4	M	2	0.850989	2.71016
R	4	M	3	0.774410	2.46627
R	4	M	4	0.876358	2.79095
R	4	M	5	0.808648	2.57531
R	4	M	6	0.911750	2.90366
R	4	M	7	0.966630	3.07844
R	7	M	1	0.796619	2.94499
R	7	M	2	0.862735	3.18941
R	7	M	3	0.799923	2.95720
R	7	M	4	0.871156	3.22054
R	7	M	5	0.746341	2.75912
R	7	M	6	0.815142	3.01346
R	7	M	7	0.829787	3.06761
R	10	M	1	0.336067	1.85161
R	10	M	2	0.365362	2.01302
R	10	M	3	0.291186	1.60433
R	10	M	4	0.340070	1.87367
R	10	M	5	0.411421	2.26678
R	10	M	6	0.455765	2.51110
R	10	M	7	0.466644	2.57104
R	14	M	1	0.370710	1.51310
R	14	M	2	0.433942	1.77119
R	14	M	3	0.437882	1.78727
R	14	M	4	0.537061	2.19209

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 3 (cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
R	14	M	5	0.801479	3.27134
R	14	M	6	0.787604	3.21471
R	14	M	7	0.705418	2.87926
R	20	F	1	0.872100	3.17705
R	20	F	2	0.917929	3.34400
R	20	F	3	0.878738	3.20123
R	20	F	4	0.990149	3.60710
R	20	F	5	0.889861	3.24175
R	20	F	6	0.882768	3.21591
R	20	F	7	0.848793	3.09214
R	22	F	1	0.508456	2.64133
R	22	F	2	0.472473	2.45441
R	22	F	3	0.415251	2.15715
R	22	F	4	0.610505	3.17145
R	22	F	5	0.578099	3.00311
R	22	F	6	0.569013	2.95591
R	22	F	7	0.547067	2.84191
R	25	F	1	0.748790	2.82562
R	25	F	2	0.785435	2.96391
R	25	F	3	0.697441	2.63185
R	25	F	4	0.771361	2.91080
R	25	F	5	0.661830	2.49747
R	25	F	6	0.679058	2.56248
R	25	F	7	0.677380	2.55615
R	31	F	1	0.548181	2.46928
R	31	F	2	0.630439	2.83982
R	31	F	3	0.608278	2.73999
R	31	F	4	0.707262	3.18586
R	31	F	5	0.673755	3.03493
R	31	F	6	0.683709	3.07977
R	31	F	7	0.675946	3.04480
R	36	F	1	0.787235	2.92652
R	36	F	2	0.853381	3.17242
R	36	F	3	0.806641	2.99867
R	36	F	4	0.935122	3.47629
R	36	F	5	0.892998	3.31970
R	36	F	6	0.910868	3.38612
R	36	F	7	0.885154	3.29053
R	38	F	1	0.765347	2.91561
R	38	F	2	0.853283	3.25060
R	38	F	3	0.779481	2.96945
R	38	F	4	0.874866	3.33282
R	38	F	5	0.755166	2.87682
R	38	F	6	0.795435	3.03023
R	38	F	7	0.809829	3.08506
V	3	M	1	0.853280	3.05288
V	3	M	2	0.917015	3.28091
V	3	M	3	0.912221	3.26376
V	3	M	4	0.860818	3.07985
V	3	M	5	0.833238	2.98117
V	3	M	6	0.907906	3.24832
V	3	M	7	0.938732	3.35861
V	5	M	1	0.778879	2.93917
V	5	M	2	0.739466	2.79044
V	5	M	3	0.731028	2.75860

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 3 (cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
V	5	M	4	0.741179	2.79690
V	5	M	5	0.723507	2.73022
V	5	M	6	0.754978	2.84897
V	5	M	7	0.786381	2.96747
V	9	M	1	0.636257	2.60761
V	9	M	2	0.536623	2.19928
V	9	M	3	0.526332	2.15710
V	9	M	4	0.534957	2.19245
V	9	M	5	0.552405	2.26395
V	9	M	6	0.551729	2.26118
V	9	M	7	0.567723	2.32673
V	13	M	1	0.659352	2.43753
V	13	M	2	0.626253	2.31517
V	13	M	3	0.660235	2.44079
V	13	M	4	0.706741	2.61272
V	13	M	5	0.741625	2.74168
V	13	M	6	0.720734	2.66445
V	13	M	7	0.707223	2.61450
V	19	F	1	0.708357	2.89717
V	19	F	2	0.703244	2.87625
V	19	F	3	0.705906	2.88714
V	19	F	4	0.708988	2.89975
V	19	F	5	0.724673	2.96390
V	19	F	6	0.693504	2.83642
V	19	F	7	0.730273	2.98680
V	21	F	1	0.862541	2.86559
V	21	F	2	0.816889	2.71392
V	21	F	3	0.844075	2.80424
V	21	F	4	0.931527	3.09477
V	21	F	5	0.981522	3.26087
V	21	F	6	0.933996	3.10298
V	21	F	7	0.880071	2.92382
V	28	F	1	0.702714	2.63682
V	28	F	2	0.717010	2.69047
V	28	F	3	0.723416	2.71451
V	28	F	4	0.756072	2.83704
V	28	F	5	0.759187	2.84873
V	28	F	6	0.756145	2.83732
V	28	F	7	0.733175	2.75112
V	30	F	1	0.609566	2.96626
V	30	F	2	0.591952	2.88055
V	30	F	3	0.572895	2.78781
V	30	F	4	0.593268	2.88695
V	30	F	5	0.611748	2.97688
V	30	F	6	0.627184	3.05199
V	30	F	7	0.647213	3.14945
V	33	F	1	0.773974	3.12086
V	33	F	2	0.832246	3.35583
V	33	F	3	0.794556	3.20385
V	33	F	4	0.873939	3.52395
V	33	F	5	0.776976	3.13297
V	33	F	6	0.696639	2.80903
V	33	F	7	0.745133	3.00457
V	37	F	1	0.761099	2.73285
V	37	F	2	0.784389	2.81648
V	37	F	3	0.790856	2.83970
V	37	F	4	0.840445	3.01776
V	37	F	5	0.864276	3.10332
V	37	F	6	0.884985	3.17769
V	37	F	7	0.946039	3.39691

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 4: Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
C	4	F	1	0.44755	1.98033
C	4	F	2	0.43685	1.93298
C	4	F	3	0.41192	1.82266
C	4	F	4	0.43283	1.91516
C	4	F	5	0.46028	2.03666
C	4	F	6	0.40659	1.79908
C	4	F	7	0.43260	1.91418
C	5	M	1	0.70463	2.77412
C	5	M	2	0.70903	2.79147
C	5	M	3	0.69970	2.75474
C	5	M	4	0.72121	2.83943
C	5	M	5	0.75986	2.99159
C	5	M	6	0.72302	2.84654
C	5	M	7	0.74716	2.94159
C	11	M	1	0.77476	3.00295
C	11	M	2	0.77526	3.00489
C	11	M	3	0.73322	2.84196
C	11	M	4	0.73623	2.85359
C	11	M	5	0.74137	2.87353
C	11	M	6	0.68561	2.65742
C	11	M	7	0.75394	2.92225
C	14	M	1	1.02257	3.07077
C	14	M	2	1.00779	3.02640
C	14	M	3	0.98042	2.94422
C	14	M	4	0.96698	2.90384
C	14	M	5	1.00190	3.00870
C	14	M	6	0.93347	2.80321
C	14	M	7	1.02760	3.08590
C	18	M	1	0.60087	2.44753
C	18	M	2	0.59849	2.43782
C	18	M	3	0.59192	2.41109
C	18	M	4	0.60471	2.46319
C	18	M	5	0.61403	2.50115
C	18	M	6	0.58359	2.37714
C	18	M	7	0.62844	2.55982
C	22	F	1	0.52225	2.33667
C	22	F	2	0.51732	2.31461
C	22	F	3	0.44370	1.98525
C	22	F	4	0.41911	1.87519
C	22	F	5	0.44620	1.99640
C	22	F	6	0.45812	2.04977
C	22	F	7	0.52041	2.32844
C	27	F	1	0.66473	2.83468
C	27	F	2	0.65116	2.77680
C	27	F	3	0.63415	2.70426
C	27	F	4	0.64173	2.73657
C	27	F	5	0.64741	2.76083
C	27	F	6	0.60942	2.59882
C	27	F	7	0.61939	2.64134
C	31	F	1	0.60483	2.25683
C	31	F	2	0.62006	2.31367
C	31	F	3	0.60806	2.26889
C	31	F	4	0.63225	2.35914
C	31	F	5	0.63883	2.38369
C	31	F	6	0.61089	2.27945

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 4 (Cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
C	31	F	7	0.682244	2.54569
C	35	F	1	0.625266	2.68931
C	35	F	2	0.640088	2.75307
C	35	F	3	0.641308	2.75831
C	35	F	4	0.669029	2.87754
C	35	F	5	0.689522	2.96568
C	35	F	6	0.667675	2.87172
C	35	F	7	0.723703	3.11270
C	37	F	1	0.409507	1.57806
C	37	F	2	0.563120	2.17002
C	37	F	3	0.529078	2.03884
C	37	F	4	0.548111	2.11218
C	37	F	5	0.595773	2.29585
C	37	F	6	0.559769	2.15711
C	37	F	7	0.599158	2.30889
F	3	F	1	0.586294	2.54357
F	3	F	2	0.625776	2.71486
F	3	F	3	0.573612	2.48855
F	3	F	4	0.574188	2.49105
F	3	F	5	0.568742	2.46743
F	3	F	6	0.577516	2.50549
F	3	F	7	0.554723	2.40661
F	7	M	1	0.282746	1.23201
F	7	M	2	0.503358	2.19328
F	7	M	3	0.420611	1.83273
F	7	M	4	0.418965	1.82556
F	7	M	5	0.380659	1.65865
F	7	M	6	0.335158	1.46039
F	7	M	7	0.292833	1.27596
F	9	M	1	0.692727	2.49182
F	9	M	2	0.719282	2.58734
F	9	M	3	0.690317	2.48315
F	9	M	4	0.723034	2.60084
F	9	M	5	0.721685	2.59599
F	9	M	6	0.742529	2.67097
F	9	M	7	0.712081	2.56144
F	15	M	1	0.593490	2.71000
F	15	M	2	0.648730	2.96224
F	15	M	3	0.657565	3.00258
F	15	M	4	0.705409	3.22105
F	15	M	5	0.665394	3.03833
F	15	M	6	0.681072	3.10992
F	15	M	7	0.673914	3.07723
F	20	M	1	0.483312	1.95277
F	20	M	2	0.523241	2.11410
F	20	M	3	0.497910	2.01176
F	20	M	4	0.476550	1.92546
F	20	M	5	0.452147	1.82686
F	20	M	6	0.451448	1.82403
F	20	M	7	0.435761	1.76065
F	24	F	1	0.651942	2.72210
F	24	F	2	0.675334	2.81977
F	24	F	3	0.631595	2.63714
F	24	F	4	0.674958	2.81820
F	24	F	5	0.671665	2.80445

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 4 (Cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
F	24	F	6	0.69987	2.92221
F	24	F	7	0.70681	2.95119
F	28	F	1	0.64193	2.58322
F	28	F	2	0.63687	2.56285
F	28	F	3	0.57505	2.31408
F	28	F	4	0.58679	2.36133
F	28	F	5	0.57309	2.30622
F	28	F	6	0.56300	2.26558
F	28	F	7	0.54979	2.21243
F	32	F	1	0.44984	2.11192
F	32	F	2	0.47119	2.21216
F	32	F	3	0.45516	2.13691
F	32	F	4	0.47962	2.25173
F	32	F	5	0.48353	2.27009
F	32	F	6	0.49633	2.33019
F	32	F	7	0.49555	2.32653
F	33	F	1	0.70844	2.81686
F	33	F	2	0.72595	2.88650
F	33	F	3	0.69300	2.75547
F	33	F	4	0.69936	2.78074
F	33	F	5	0.69855	2.77753
F	33	F	6	0.71147	2.82889
F	33	F	7	0.72449	2.88070
F	40	F	1	0.85387	3.24050
F	40	F	2	0.87914	3.33639
F	40	F	3	0.85653	3.25059
F	40	F	4	0.90948	3.45155
F	40	F	5	0.89731	3.40535
F	40	F	6	0.88820	3.37077
F	40	F	7	0.84493	3.20658
R	1	F	1	0.59301	2.38156
R	1	F	2	0.52217	2.09708
R	1	F	3	0.53624	2.15356
R	1	F	4	0.50398	2.02402
R	1	F	5	0.54905	2.20504
R	1	F	6	0.54155	2.17490
R	1	F	7	0.51086	2.05164
R	8	M	1	0.74828	3.74141
R	8	M	2	0.59359	2.96795
R	8	M	3	0.56360	2.81802
R	8	M	4	0.53765	2.68824
R	8	M	5	0.57057	2.85287
R	8	M	6	0.55411	2.77053
R	8	M	7	0.58475	2.92373
R	12	M	1	1.03293	3.70889
R	12	M	2	0.92014	3.30391
R	12	M	3	0.91420	3.28258
R	12	M	4	0.86247	3.09683
R	12	M	5	0.96546	3.46665
R	12	M	6	0.92972	3.33831
R	12	M	7	0.96934	3.48059
R	13	M	1	0.60391	2.77021
R	13	M	2	0.53012	2.43174
R	13	M	3	0.53623	2.45978
R	13	M	4	0.52584	2.41209

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 4 (Cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
R	13	M	5	0.571285	2.62058
R	13	M	6	0.534896	2.45365
R	13	M	7	0.556272	2.55171
R	19	M	1	0.768008	3.05371
R	19	M	2	0.701048	2.78747
R	19	M	3	0.671509	2.67001
R	19	M	4	0.655083	2.60470
R	19	M	5	0.747308	2.97140
R	19	M	6	0.692098	2.75188
R	19	M	7	0.725883	2.88621
R	21	F	1	0.614995	3.21146
R	21	F	2	0.491735	2.56781
R	21	F	3	0.331471	1.73092
R	21	F	4	0.284349	1.48485
R	21	F	5	0.284732	1.48685
R	21	F	6	0.336324	1.75626
R	21	F	7	0.357485	1.86676
R	25	F	1	0.862300	3.36836
R	25	F	2	0.716522	2.79891
R	25	F	3	0.724403	2.82970
R	25	F	4	0.700490	2.73629
R	25	F	5	0.748052	2.92208
R	25	F	6	0.722086	2.82065
R	25	F	7	0.764899	2.98789
R	30	F	1	0.724648	2.93380
R	30	F	2	0.639017	2.58711
R	30	F	3	0.640698	2.59392
R	30	F	4	0.615758	2.49295
R	30	F	5	0.678609	2.74740
R	30	F	6	0.636116	2.57537
R	30	F	7	0.651718	2.63854
R	34	F	1	0.691878	2.67651
R	34	F	2	0.580168	2.24436
R	34	F	3	0.568838	2.20053
R	34	F	4	0.556756	2.15380
R	34	F	5	0.616450	2.38472
R	34	F	6	0.598478	2.31520
R	34	F	7	0.608730	2.35486
R	39	F	1	0.764063	3.13141
R	39	F	2	0.667970	2.73758
R	39	F	3	0.667408	2.73528
R	39	F	4	0.651937	2.67187
R	39	F	5	0.716559	2.93672
R	39	F	6	0.690642	2.83050
R	39	F	7	0.686752	2.81456
V	2	F	1	0.422228	2.03483
V	2	F	2	0.480444	2.31539
V	2	F	3	0.406666	1.95984
V	2	F	4	0.423256	2.03979
V	2	F	5	0.407707	1.96485
V	2	F	6	0.363224	1.75048
V	2	F	7	0.352756	1.70003
V	6	M	1	0.457584	2.09421
V	6	M	2	0.408725	1.87060
V	6	M	3	0.375236	1.71733

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 4 (Cont): Individual data for intake trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	SEX	DAY	INTAKE	PBDWT
V	6	M	4	0.402439	1.84182
V	6	M	5	0.406461	1.86023
V	6	M	6	0.376324	1.72231
V	6	M	7	0.385437	1.76401
V	10	M	1	0.837883	2.96596
V	10	M	2	0.772108	2.73313
V	10	M	3	0.661508	2.34162
V	10	M	4	0.716929	2.53780
V	10	M	5	0.713824	2.52681
V	10	M	6	0.629409	2.22800
V	10	M	7	0.615790	2.17979
V	16	M	1	0.572950	2.59841
V	16	M	2	0.556671	2.52459
V	16	M	3	0.475465	2.15631
V	16	M	4	0.852700	3.86712
V	16	M	5	0.785732	3.56341
V	16	M	6	0.652807	2.96058
V	16	M	7	0.618894	2.80678
V	17	M	1	0.683150	2.38863
V	17	M	2	0.696351	2.43479
V	17	M	3	0.626982	2.19224
V	17	M	4	0.703295	2.45907
V	17	M	5	0.730860	2.55546
V	17	M	6	0.704668	2.46388
V	17	M	7	0.729106	2.54932
V	23	F	1	0.623966	2.59445
V	23	F	2	0.577422	2.40092
V	23	F	3	0.455307	1.89317
V	23	F	4	0.469652	1.95282
V	23	F	5	0.499194	2.07565
V	23	F	6	0.475783	1.97831
V	23	F	7	0.519789	2.16129
V	26	F	1	0.620658	3.02023
V	26	F	2	0.603978	2.93907
V	26	F	3	0.552755	2.68981
V	26	F	4	0.604259	2.94043
V	26	F	5	0.614721	2.99134
V	26	F	6	0.591492	2.87831
V	26	F	7	0.632522	3.07796
V	29	F	1	0.753214	3.01889
V	29	F	2	0.792159	3.17499
V	29	F	3	0.718716	2.88062
V	29	F	4	0.833467	3.34055
V	29	F	5	0.776547	3.11241
V	29	F	6	0.717681	2.87648
V	29	F	7	0.758791	3.04125
V	36	F	1	0.738308	2.91821
V	36	F	2	0.729357	2.88284
V	36	F	3	0.674700	2.66680
V	36	F	4	0.785650	3.10534
V	36	F	5	0.796531	3.14835
V	36	F	6	0.776650	3.06976
V	36	F	7	0.825593	3.26321
V	38	F	1	0.601736	2.81185
V	38	F	2	0.608376	2.84288
V	38	F	3	0.600425	2.80573
V	38	F	4	0.700150	3.27173
V	38	F	5	0.710340	3.31935
V	38	F	6	0.690251	3.22547
V	38	F	7	0.710165	3.31853

VARIETY=cultivar SHEEP=lamb number SEX=sex of lamb
DAY=day of intake trial INTAKE=dry matter intake (kg day^{-1})
PBDWT=dry matter intake as percent of body weight (%)

APPENDIX 5: Individual data for digestibility trial comparing initial growth of RCG and timothy cultivars. Study II. Experiment I.

VARIETY	SHEEP	INTAKE	DDM	PROTDIG	ADFDIG	NDFDIG
C	2	525.621	62.8268	69.3877	58.7609	66.5603
C	8	447.093	61.9336	69.0297	58.4664	66.2362
C	12	678.819	61.9023	69.1537	55.2154	63.9973
C	15	523.980	62.7263	71.2035	58.8469	66.3830
F	1	594.214	58.2313	74.2564	52.0835	56.9878
F	6	628.314	59.3376	73.2604	53.6752	59.0739
F	11	486.010	57.0449	74.6286	50.1710	55.6437
F	16	657.203	58.5593	72.9351	51.9211	57.4164
R	4	657.822	59.8795	70.1799	54.1908	63.0877
R	7	705.848	60.4683	74.5976	54.2936	60.1722
R	10	344.072	59.7432	73.8449	56.8087	62.4017
R	14	510.318	61.6463	73.9733	56.9396	63.0540
V	3	780.939	58.6834	76.6845	50.5765	57.1026
V	5	688.413	59.5541	77.3610	52.8747	59.6776
V	9	473.595	57.2748	77.2219	50.2627	57.5338
V	13	631.981	59.7485	75.8768	53.6062	60.8360

VARIETY=cultivar SHEEP=lamb number INTAKE=averge daily DM intake (g)
DDM=DM digestibility (%) PROTDIG=CP digestibility (%)
ADFDIG=ADF digestibility (%) NDFDIG=NDF digestibility (%)

APPENDIX 6: Individual data for digestibility trial comparing regrowth of RCG and timothy cultivars. Study II. Experiment II.

VARIETY	SHEEP	INTAKE	DDM	PROTDIG	ADFDIG	NDFDIG
C	5	654.950	57.4970	61.2674	50.1482	58.9054
C	11	679.323	56.3708	62.7590	48.0024	56.7005
C	14	901.187	60.0539	66.1112	50.3860	59.8767
C	18	544.259	59.9785	65.2520	51.7962	60.9120
F	7	289.490	52.5693	72.7485	49.8964	54.6894
F	9	596.824	54.2923	74.1033	47.8912	52.2187
F	15	542.327	56.3518	72.7665	50.4253	56.1500
F	20	417.064	54.4474	71.9775	49.2822	55.3528
R	8	536.422	61.8171	76.9341	58.6461	62.6020
R	12	847.865	61.1869	76.4869	55.1935	60.6364
R	13	474.420	60.1950	77.0920	57.2509	61.9633
R	19	655.766	59.4276	74.3779	53.8053	60.4424
V	6	366.732	54.9070	72.7480	49.6274	55.7807
V	10	619.896	57.8104	74.5525	52.1106	57.6095
V	16	502.288	50.7209	71.1137	41.5389	49.8705
V	17	599.995	57.4665	75.2506	48.3997	55.3500

VARIETY=cultivar SHEEP=lamb number INTAKE=averge daily DM intake (g)
DDM=DM digestibility (%) PROTDIG=CP digestibility (%)
ADFDIG=ADF digestibility (%) NDFDIG=NDF digestibility (%)

APPENDIX 7: Analysis of variance for intake trial comparing initial growth and regrowth of RCG and timothy cultivars. Study II.

Parameter	Source	df	Anova SS	F value	Pr > F
Initial Growth (Experiment I)					
Intake	cultivar	3	0.3627	0.76	0.5243
	sex	1	0.2063	1.30	0.2630
	cultivar*sex	3	0.0482	0.10	0.9588
	sheep(cultivar*sex)	32	5.0849	46.99	0.0001
	day	6	0.1766	8.70	0.0001
	cultivar*day	18	0.0947	1.56	0.0751
	sex*day	6	0.0652	3.22	0.0049
	cultivar*sex*day	18	0.0753	1.24	0.2347
	Error	192	0.6493		
Intake as a percent of body weight	cultivar	3	2.9718	1.09	0.3665
	sex	1	5.6830	6.27	0.0176
	cultivar*sex	3	0.5647	0.21	0.8904
	sheep(cultivar*sex)	32	29.0212	15.70	0.0001
	day	6	3.1442	9.07	0.0001
	cultivar*day	18	1.6488	1.59	0.0668
	sex*day	6	1.0599	3.06	0.0070
	cultivar*sex*day	18	1.2691	1.22	0.2477
	Error	192	11.0896		
Regrowth (Experiment II)					
Intake	cultivar	3	0.0572	0.14	0.9363
	sex	1	0.1616	1.17	0.2866
	cultivar*sex	3	0.8215	1.99	0.1352
	sheep(cultivar*sex)	32	4.4030	164.35	0.0001
	day	6	0.0682	82.57	0.0001
	cultivar*day	18	0.1642	6.82	0.0001
	sex*day	6	0.0063	5.48	0.0001
	cultivar*sex*day	18	0.0229	0.63	0.7047
	Error	192	0.3199		
Intake as a percent of body weight	cultivar	3	0.9270	0.25	0.8637
	sex	1	0.1945	0.15	0.6967
	cultivar*sex	3	10.2580	2.75	0.0608
	sheep(cultivar*sex)	32	40.2333	37.43	0.0001
	day	6	1.2123	6.01	0.0001
	cultivar*day	18	3.0895	5.11	0.0001
	sex*day	6	0.1311	0.65	0.6899
	cultivar*sex*day	18	0.3946	0.65	0.8539
	Error	192	6.4498		

APPENDIX 8: Analysis of variance for digestibility trial comparing initial growth and regrowth of RCG and timothy cultivars. Study II.

Parameter	Source	df	Anova SS	F value	Pr > F
Initial Growth (Experiment I)					
Dry matter	cultivar	3	40.0477	16.81	0.0001
	error	12	9.5291		
Acid detegerent fiber	cultivar	3	102.2163	13.40	0.0004
	error	12	30.5158		
Neutral detergent fiber	cultivar	3	101.5700	21.99	0.0001
	error	12			
Crude protein	cultivar	3	172.4188	27.13	0.0001
	error	12	25.4247		
Regrowth (Experiment II)					
Dry matter	cultivar	3	100.8996	7.65	0.0040
	error	12	52.7597		
Acid detegerent fiber	cultivar	3	345.8319	42.67	0.0001
	error	12	32.4178		
Neutral detergent fiber	cultivar	3	160.9058	7.47	0.0044
	error	12	86.2030		
Crude protein	cultivar	3	137.3568	10.00	0.0014
	error	12	54.9626		

APPENDIX 9: Weekly data on alkaloid and nutrient concentration (DM basis) in each RCG paddock during grazing season. Study III.

VARIETY	PEN	WEEK	GRAM	HORD	CP	ADF	NDF
FRONTIER	2	1	0.48201	1.87982	28.6005	29.5207	55.3606
FRONTIER	2	2	0.69332	1.93169	28.8913	25.4885	55.6202
FRONTIER	2	3	0.33606	2.62126	24.0440	30.2165	60.8424
FRONTIER	2	4	1.37963	2.94084	18.2603	31.3932	61.0916
FRONTIER	2	5	2.45453	2.93557	15.6638	36.0484	64.6754
FRONTIER	2	6	2.33236	3.27884	19.5810	31.9367	58.7751
FRONTIER	2	7	2.97152	2.78345	20.7100	31.8296	61.9224
FRONTIER	2	8	3.17325	3.82306	19.8152	35.3965	65.0333
FRONTIER	2	9	2.88611	3.42957	19.7318	33.8519	62.8755
FRONTIER	2	10	3.72050	3.55663	18.5382	32.8297	58.2993
FRONTIER	2	11	2.48256	3.55422	20.8102	32.9195	62.7790
FRONTIER	2	12	2.29737	3.65617	18.6437	34.1442	63.0355
FRONTIER	2	13	2.60628	2.35403	15.7727	34.6999	63.2820
FRONTIER	2	14	1.80769	2.37500	13.0481	36.0623	63.5670
FRONTIER	2	15	1.16114	1.87305	14.1509	33.9521	60.9454
FRONTIER	2	16	2.08121	1.93532	11.8699	33.5772	60.9146
FRONTIER	5	1	0.35274	1.64788	28.6836	27.0596	55.1409
FRONTIER	5	2	0.51258	2.33313	27.5391	26.2515	56.2105
FRONTIER	5	3	0.17628	2.01809	24.0514	29.2176	60.1929
FRONTIER	5	4	0.26285	2.08161	20.1952	29.5474	58.9876
FRONTIER	5	5	1.46458	2.58750	14.3707	38.5564	68.2827
FRONTIER	5	6	4.18881	3.34505	11.8158	36.9393	64.3378
FRONTIER	5	7	3.70758	3.04376	16.1231	36.7279	65.2592
FRONTIER	5	8	3.35339	2.96857	17.1361	36.4785	66.0491
FRONTIER	5	9	2.48789	2.46099	17.4429	36.5627	66.8554
FRONTIER	5	10	3.18888	3.19628	18.4244	31.1078	61.8541
FRONTIER	5	11	0.83082	2.03508	16.8260	34.7401	64.8972
FRONTIER	5	12	3.75916	3.13263	18.0112	33.7468	62.2412
FRONTIER	5	13	3.40676	3.24695	17.0499	35.4032	62.4245
FRONTIER	5	14	2.41873	2.97690	15.4374	34.5731	61.4328
FRONTIER	5	15	2.09470	2.06382	11.8440	37.9635	61.0233
FRONTIER	5	16	2.02740	2.18076	13.3969	33.1371	61.2301
RIVAL	3	1	0.59291	1.15405	29.7672	25.9769	55.2530
RIVAL	3	2	0.78075	2.07243	25.1132	24.4285	56.4771
RIVAL	3	3	1.48089	3.16037	27.0195	26.5462	58.9222
RIVAL	3	4	1.05102	3.02721	18.8443	30.1572	57.7225
RIVAL	3	5	4.01475	4.49153	16.6307	35.6249	64.2672
RIVAL	3	6	9.22353	6.11446	18.9401	32.6561	61.3566
RIVAL	3	7	6.12041	4.30672	17.2512	36.9356	66.1552
RIVAL	3	8	7.12301	5.06679	18.4843	34.8111	65.3070
RIVAL	3	9	6.10866	4.13468	20.5499	32.8194	62.9326
RIVAL	3	10	5.32187	3.93659	19.9215	31.0544	60.2224
RIVAL	3	11	5.17421	3.70436	19.5093	33.3114	62.8985
RIVAL	3	12	3.38922	3.68340	17.2718	34.8669	64.2603
RIVAL	3	13	3.22897	3.50387	15.1316	33.4359	61.1418
RIVAL	3	14	4.18333	2.84185	14.1948	33.7644	61.7301
RIVAL	3	15	2.41751	2.94148	12.2819	34.2901	61.7343
RIVAL	3	16	2.35648	2.33099	10.8448	34.1592	62.5406
RIVAL	7	1	0.40191	1.00097	25.7710	26.6013	55.8443
RIVAL	7	2	0.26056	2.06346	27.1845	29.2034	56.4431
RIVAL	7	3	0.50435	2.14428	25.3205	27.1688	57.2115
RIVAL	7	4	1.55687	3.10817	21.5380	26.7324	59.6551
RIVAL	7	5	1.55278	2.98406	14.2703	30.2270	67.1027
RIVAL	7	6	3.08001	2.95075	16.6811	36.2511	65.2802
RIVAL	7	7	2.83169	3.09683	17.8282	34.8028	64.4192

VARIETY=cultivar PEN=paddock WEEK=week of grazing season GRAM=gramine (mg g^{-1}) HORD=hordenine (mg g^{-1}) CP=CP (%) ADF=ADF (%) NDF=NDF (%)

APPENDIX 9 (cont): Weekly data on alkaloid and nutrient concentration (DM basis) in each RCG paddock during grazing season. Study III.

VARIETY	PEN	WEEK	GRAM	HORD	CP	ADF	NDF
RIVAL	7	8	5.89124	3.87540	18.4778	34.8634	67.9547
RIVAL	7	9	5.48490	4.05723	20.1420	33.3262	62.1357
RIVAL	7	10	4.94283	3.67672	17.7063	33.2684	61.1219
RIVAL	7	11	4.09287	3.13793	16.2298	34.4692	65.0502
RIVAL	7	12	4.27933	3.07643	18.4394	33.6696	65.7400
RIVAL	7	13	3.13103	2.39545	14.2450	34.2555	62.2386
RIVAL	7	14	4.50081	3.34700	14.9378	35.9793	62.1653
RIVAL	7	15	2.10221	2.16739	10.3159	35.5133	63.2719
RIVAL	7	16	3.39066	2.25942	9.7836	30.0823	61.6479
VENTURE	4	1	0.74059	1.32802	29.5249	25.8921	55.9544
VENTURE	4	2	0.71805	1.96508	27.0071	26.3938	55.3970
VENTURE	4	3	0.40160	2.17796	22.3707	29.2967	61.2762
VENTURE	4	4	0.59925	2.67908	16.2411	36.9001	65.0916
VENTURE	4	5	1.14437	2.20767	12.7306	40.4898	68.9934
VENTURE	4	6	2.87141	2.48127	15.9538	37.0188	64.9059
VENTURE	4	7	2.80541	2.81514	15.7362	38.3658	67.0631
VENTURE	4	8	4.56506	3.42119	18.6344	34.4301	64.4409
VENTURE	4	9	2.78925	1.97893	18.2179	34.1170	63.9291
VENTURE	4	10	3.33333	2.49513	18.9442	33.0654	60.0478
VENTURE	4	11	2.51270	2.24704	17.0078	37.2770	66.9038
VENTURE	4	12	2.66845	1.84467	16.7117	37.0446	65.5713
VENTURE	4	13	2.26893	2.11290	16.7127	31.2274	59.7048
VENTURE	4	14	1.40947	1.37657	12.3638	39.8414	67.7102
VENTURE	4	15	1.51208	1.57922	12.7254	36.6261	63.2523
VENTURE	4	16	1.55109	1.64287	11.1801	37.7762	64.9017
VENTURE	6	1	1.80852	1.81168	27.3980	27.7317	59.2636
VENTURE	6	2	0.42719	0.90067	27.7686	24.7262	57.5396
VENTURE	6	3	0.62826	1.72932	24.0387	31.9549	60.9452
VENTURE	6	4	0.55326	2.11522	18.3726	29.5396	64.9143
VENTURE	6	5	1.44128	1.91826	15.5459	35.9135	67.1351
VENTURE	6	6	3.73290	3.78318	15.5627	34.6473	65.5035
VENTURE	6	7	2.87086	3.14621	16.0876	34.8835	65.9581
VENTURE	6	8	4.90739	3.82235	17.7594	32.9864	65.9513
VENTURE	6	9	2.71582	2.83744	16.7093	35.0395	65.7603
VENTURE	6	10	3.35797	3.36942	18.2550	32.0714	61.8908
VENTURE	6	11	2.24971	3.19907	17.0155	35.1424	66.8753
VENTURE	6	12	3.17326	2.11551	16.6989	34.9570	64.9462
VENTURE	6	13	2.33085	2.01683	13.6349	35.3134	63.4817
VENTURE	6	14	1.81137	1.81137	11.9848	36.3761	64.3618
VENTURE	6	15	1.79620	1.78998	12.7708	36.4583	65.6667
VENTURE	6	16	1.75695	1.76002	8.8543	36.4644	66.8293

VARIETY=cultivar PEN=paddock WEEK=week of grazing season
GRAM=gramine (mg g^{-1}) HORD=hordenine (mg g^{-1}) CP=CP (%) ADF=ADF (%)
NDF=NDF (%)

APPENDIX 10: Determination of mineral content of forage (DM basis) in RCG paddocks on sampling dates during grazing season. Study III.

VARIETY	WEEK	PEN	CA	P	MG	CU	ZN	S
FRONTIER	2	2	2.90828	5.03356	2.68456	10.4027	34.8993	2.85235
RIVAL	2	3	4.49236	4.60467	3.59389	11.3432	31.7835	3.24573
VENTURE	2	4	2.80647	5.27616	2.58195	10.6646	31.6569	2.51459
FRONTIER	2	5	2.91774	4.82550	3.02996	9.4266	35.2373	3.91651
VENTURE	2	6	3.48393	5.50686	3.03439	10.3394	31.1306	3.31535
RIVAL	2	7	3.14007	4.48581	3.14007	9.6445	27.8120	2.92699
FRONTIER	5	2	2.10807	4.10518	2.99567	6.6571	33.1743	3.22867
RIVAL	5	3	2.66016	3.65773	2.66016	5.0986	25.7149	2.77100
VENTURE	5	4	1.88241	3.54335	2.65751	5.0936	29.6756	2.93434
FRONTIER	5	5	1.98807	3.42390	2.98211	7.4000	35.8957	3.41286
VENTURE	5	6	2.09505	3.52850	3.30797	6.7262	23.9277	2.51406
RIVAL	5	7	1.97998	3.18997	2.96997	6.7099	28.1597	2.45298
FRONTIER	8	2	3.37176	3.69806	3.69806	4.4594	23.6023	3.15423
RIVAL	8	3	3.71951	2.95372	4.48529	3.8289	17.5036	2.74587
VENTURE	8	4	3.16871	3.49650	4.15210	5.2448	28.6276	2.96110
FRONTIER	8	5	2.92145	2.70504	3.57066	3.3543	27.5914	3.19195
VENTURE	8	6	2.94214	2.94214	3.59595	4.3587	20.7039	3.51967
RIVAL	8	7	3.04348	3.15217	3.80435	3.8043	26.5217	2.89130
FRONTIER	10	2	3.47080	3.77705	3.57289	6.9212	30.2470	3.36872
RIVAL	10	3	4.37685	2.95437	4.59569	6.8935	16.9603	3.35923
VENTURE	10	4	4.46380	3.70169	4.13718	5.5525	26.1296	2.71094
FRONTIER	10	5	3.25733	2.82302	4.99457	6.0803	22.6927	3.04017
VENTURE	10	6	3.37727	3.26833	3.48622	3.5952	22.5515	3.25744
RIVAL	10	7	3.47411	2.60558	3.69124	4.8855	22.7988	3.25698
FRONTIER	13	2	3.22789	2.79750	3.55068	4.6266	19.0445	2.87282
RIVAL	13	3	4.20803	2.15796	3.88433	3.4527	11.8688	3.15063
VENTURE	13	4	4.11523	2.59909	4.33182	3.6820	18.6268	2.41499
FRONTIER	13	5	3.86723	2.25588	3.65238	3.5450	18.5842	3.92094
VENTURE	13	6	4.08383	2.36432	4.08383	3.4390	16.4428	3.10586
RIVAL	13	7	4.09306	2.26196	4.09306	3.5545	15.9414	3.16674
FRONTIER	16	2	3.45528	2.94715	3.65854	4.4004	17.7541	2.95732
RIVAL	16	3	4.06174	2.23396	3.45248	3.0361	9.1186	2.80260
VENTURE	16	4	3.76744	2.44374	3.66561	6.1705	24.9771	3.53325
FRONTIER	16	5	3.55222	2.23282	3.55222	4.9528	15.5689	3.44058
VENTURE	16	6	2.74474	2.43977	2.74474	3.7918	21.7546	2.88706
RIVAL	16	7	2.64147	1.52393	3.86061	4.1146	11.7647	3.21040

VARIETY=cultivar WEEK=week of grazing season PEN=paddock
CA=Ca (g kg^{-1}) P=P (g kg^{-1}) MG=Mg (g kg^{-1}) CU=Cu (mg kg^{-1})
ZN=Zn (mg kg^{-1}) S=S (g kg^{-1})

APPENDIX 11: Individual data of lamb productivity during period I of grazing season. Study III.

VARIETY	PEN	SHEEP	SEX	INT1	FIN1	TOTAL1	ADG1
F	2	31	M	17.7273	23.6364	5.9091	0.140693
F	2	71	F	15.9091	24.5455	8.6364	0.205628
F	2	156	M	18.6364	25.4545	6.8182	0.162338
F	2	164	M	19.5455	20.9091	1.3636	0.032468
F	2	209	F	19.5455	20.0000	0.4545	0.016234
F	2	230	F	18.6364	20.0000	1.3636	0.048701
F	2	233	M	18.6364	20.0000	1.3636	0.048701
F	2	237	F	17.2727	17.7273	0.4545	0.016234
F	2	260	M	19.0909	20.9091	1.8182	0.064935
F	5	52	M	17.2727	22.2727	5.0000	0.119048
F	5	56	F	17.2727	23.1818	5.9091	0.140693
F	5	93	F	15.4545	20.9091	5.4545	0.129870
F	5	113	M	15.4545	20.4545	5.0000	0.119048
F	5	152	M	16.8182	22.7273	5.9091	0.140693
F	5	196	F	16.3636	17.7273	1.3636	0.048701
F	5	212	F	17.7273	17.2727	-0.4545	-0.016234
F	5	235	M	20.4545	22.2727	1.8182	0.064935
F	5	251	F	12.7273	16.8182	4.0909	0.146104
F	5	255	M	17.2727	15.9091	-1.3636	-0.048701
R	3	64	F	19.5455	25.9091	6.3636	0.151515
R	3	114	F	17.2727	24.5455	7.2727	0.173160
R	3	162	M	16.3636	24.5455	8.1818	0.194805
R	3	204	F	25.0000	27.7273	2.7273	0.097403
R	3	213	F	15.4545	18.1818	2.7273	0.097403
R	3	215	F	16.8182	20.0000	3.1818	0.113636
R	3	249	M	21.3636	20.4545	-0.9091	-0.032468
R	3	253	M	18.6364	24.0909	5.4545	0.194805
R	7	7	M	19.0909	22.7273	3.6364	0.086580
R	7	97	M	17.7273	27.7273	10.0000	0.238095
R	7	117	F	17.7273	22.2727	4.5455	0.108225
R	7	124	F	20.0000	28.1818	8.1818	0.194805
R	7	126	M	23.1818	29.0909	5.9091	0.140693
R	7	185	M	20.9091	25.0000	4.0909	0.146104
R	7	186	F	14.5455	17.7273	3.1818	0.113636
R	7	194	F	20.0000	25.4545	5.4545	0.194805
R	7	239	M	14.5455	17.7273	3.1818	0.113636
R	7	252	F	15.4545	19.5455	4.0909	0.146104
V	4	13	M	19.0909	26.3636	7.2727	0.173160
V	4	111	M	19.5455	26.3636	6.8182	0.162338
V	4	127	F	14.5455	22.7273	8.1818	0.194805
V	4	160	M	18.1818	26.3636	8.1818	0.194805
V	4	163	F	19.0909	28.6364	9.5455	0.227273
V	4	217	M	19.0909	22.2727	3.1818	0.113636
V	4	218	F	20.4545	25.9091	5.4545	0.194805
V	4	240	F	15.9091	20.0000	4.0909	0.146104
V	4	256	F	17.7273	20.9091	3.1818	0.113636
V	4	259	M	19.0909	21.8182	2.7273	0.097403
V	6	28	F	20.9091	31.3636	10.4545	0.248918
V	6	55	M	14.5455	23.6364	9.0909	0.216450
V	6	65	F	17.2727	28.1818	10.9091	0.259740
V	6	68	M	19.5455	30.9091	11.3636	0.270563
V	6	100	M	17.2727	25.4545	8.1818	0.194805
V	6	181	M	14.0909	18.1818	4.0909	0.146104
V	6	193	F	17.7273	24.0909	6.3636	0.227273
V	6	205	M	21.3636	25.4545	4.0909	0.146104
V	6	241	F	20.9091	26.3636	5.45455	0.194805
V	6	250	F	15.9091	17.2727	1.36364	0.048701

VARIETY=cultivar PEN=paddock INT1=initial weight (kg)
FIN1=final weight (kg) TOTAL1=weight gain (kg) ADG1=ADG (kg day^{-1})

APPENDIX 12: Individual data of lamb productivity during period II of grazing season. Study III.

VARIETY	PEN	SHEEP	SEX	INT2	FIN2	TOTAL2	ADG2
F	2	31	M	26.3636	35.0000	8.63636	0.205628
F	2	71	F	26.8182	32.7273	5.90909	0.140693
F	2	156	M	27.7273	33.1818	5.45455	0.129870
F	2	164	M	23.1818	25.0000	1.81818	0.043290
F	2	209	F	23.1818	29.5455	6.36364	0.151515
F	2	230	F	23.6364	29.0909	5.45455	0.129870
F	2	233	M	21.3636	27.2727	5.90909	0.140693
F	2	237	F	20.0000	24.5455	4.54545	0.108225
F	2	260	M	23.1818	29.5455	6.36364	0.151515
F	5	52	M	24.0909	30.0000	5.90909	0.140693
F	5	56	F	25.9091	29.5455	3.63636	0.086580
F	5	93	F	24.5455	30.9091	6.36364	0.151515
F	5	113	M	21.3636	25.9091	4.54545	0.108225
F	5	152	M	26.3636	32.7273	6.36364	0.151515
F	5	196	F	17.7273	19.0909	1.36364	0.032468
F	5	212	F	20.9091	25.9091	5.00000	0.119048
F	5	235	M	24.0909	30.0000	5.90909	0.140693
F	5	251	F	19.0909	22.7273	3.63636	0.086580
F	5	255	M	17.2727	22.7273	5.45455	0.129870
R	3	64	F	27.2727	30.4545	3.18182	0.075758
R	3	114	F	24.5455	31.3636	6.81818	0.162338
R	3	162	M	25.4545	32.7273	7.27273	0.173160
R	3	204	F	24.5455	25.9091	1.36364	0.032468
R	3	213	F	19.5455	27.2727	7.72727	0.183983
R	3	215	F	19.0909	22.2727	3.18182	0.075758
R	3	249	M	19.5455	25.4545	5.90909	0.140693
R	3	253	M	20.9091	26.8182	5.90909	0.140693
R	7	7	M	25.0000	30.4545	5.45455	0.129870
R	7	97	M	28.6364	34.0909	5.45455	0.129870
R	7	117	F	23.1818	28.6364	5.45455	0.129870
R	7	124	F	30.4545	34.5455	4.09091	0.097403
R	7	126	M	30.4545	32.7273	2.27273	0.054113
R	7	185	M	27.7273	34.5455	6.81818	0.162338
R	7	186	F	20.0000	26.3636	6.36364	0.151515
R	7	194	F	28.6364	34.5455	5.90909	0.140693
R	7	239	M	19.5455	26.8182	7.27273	0.173160
R	7	252	F	20.9091	27.7273	6.81818	0.162338
V	4	13	M	27.2727	31.8182	4.54545	0.108225
V	4	111	M	25.4545	30.0000	4.54545	0.108225
V	4	127	F	22.7273	26.8182	4.09091	0.097403
V	4	160	M	26.8182	32.7273	5.90909	0.140693
V	4	163	F	30.0000	36.3636	6.36364	0.151515
V	4	217	M	23.6364	26.3636	2.72727	0.064935
V	4	218	F	27.2727	35.0000	7.72727	0.183983
V	4	240	F	21.8182	25.4545	3.63636	0.086580
V	4	256	F	21.8182	27.2727	5.45455	0.129870
V	4	259	M	20.0000	21.3636	1.36364	0.032468
V	6	28	F	34.0909	38.6364	4.54545	0.108225
V	6	55	M	25.4545	30.9091	5.45455	0.129870
V	6	65	F	27.2727	31.3636	4.09091	0.097403
V	6	68	M	30.9091	38.6364	7.72727	0.183983
V	6	100	M	27.7273	35.0000	7.27273	0.173160
V	6	181	M	19.5455	24.0909	4.54545	0.108225
V	6	193	F	26.3636	32.2727	5.90909	0.140693
V	6	205	M	26.8182	33.1818	6.36364	0.151515
V	6	241	F	27.2727	30.9091	3.63636	0.086580
V	6	250	F	18.6364	23.6364	5.00000	0.119048

VARIETY=cultivar PEN=paddock INT1=initial weight (kg)
FIN1=final weight (kg) TOTAL1=weight gain (kg) ADG1=ADG (kg day^{-1})

APPENDIX 13: Data on estimation of animal intake (DM basis) using samples taken in and out of exclusion cages in each paddock during grazing season. Study III.

VARIETY	PEN	WEEK	DMYLDI	DMYLDO	INTAKE
FRONT	2	1	1214.33	1214.33	0.00
FRONT	2	2	1535.68	1339.34	196.34
FRONT	2	3	2557.65	2049.82	507.83
FRONT	2	4	2007.53	812.09	1195.44
FRONT	2	5	667.28	584.28	83.00
FRONT	2	6	903.72	1095.61	-191.89
FRONT	2	7	809.78	933.73	-123.95
FRONT	2	8	983.18	1035.05	-51.87
FRONT	2	9	1383.78	1383.78	0.00
FRONT	2	10	1483.51	1483.51	0.00
FRONT	2	11	1391.81	1511.85	-120.04
FRONT	2	12	1445.17	1375.57	69.60
FRONT	2	13	2028.69	2053.34	-24.65
FRONT	2	14	1974.69	1562.82	411.87
FRONT	2	15	1932.88	1771.81	161.07
FRONT	2	16	1745.47	1639.92	105.55
FRONT	5	1	1006.37	1006.37	0.00
FRONT	5	2	1326.18	1245.51	80.67
FRONT	5	3	2305.00	1954.74	350.26
FRONT	5	4	2569.86	1525.12	1044.74
FRONT	5	5	1183.49	602.08	581.41
FRONT	5	6	849.04	692.91	156.13
FRONT	5	7	525.29	843.92	-318.63
FRONT	5	8	1153.80	884.73	269.07
FRONT	5	9	1529.52	1529.52	0.00
FRONT	5	10	1666.47	1666.47	0.00
FRONT	5	11	1575.51	1232.94	342.57
FRONT	5	12	1569.16	1197.57	371.59
FRONT	5	13	1370.06	1205.66	164.40
FRONT	5	14	1715.36	1754.46	-39.10
FRONT	5	15	1652.05	1494.12	157.93
FRONT	5	16	1834.75	1331.47	503.28
RIVAL	3	1	892.10	892.10	0.00
RIVAL	3	2	1184.49	1270.56	-86.07
RIVAL	3	3	1971.12	2072.15	-101.03
RIVAL	3	4	1587.95	1314.47	273.48
RIVAL	3	5	707.45	505.11	202.34
RIVAL	3	6	744.63	624.34	120.29
RIVAL	3	7	888.63	793.01	95.62
RIVAL	3	8	870.15	532.91	337.24
RIVAL	3	9	1503.14	1503.14	0.00
RIVAL	3	10	1755.07	1755.07	0.00
RIVAL	3	11	1546.26	1734.48	-188.22
RIVAL	3	12	1662.21	1820.86	-158.65
RIVAL	3	13	2163.61	1744.47	419.14
RIVAL	3	14	2021.76	1740.67	281.09
RIVAL	3	15	1985.12	1647.61	337.51
RIVAL	3	16	2154.91	2192.02	-37.11
RIVAL	7	1	767.54	767.54	0.00
RIVAL	7	2	893.68	883.13	10.55
RIVAL	7	3	1349.08	1152.85	196.23
RIVAL	7	4	1431.57	1054.22	377.35
RIVAL	7	5	614.57	534.74	79.83
RIVAL	7	6	923.85	445.57	478.28
RIVAL	7	7	415.93	477.26	-61.33

VARIETY=cultivar PEN=paddock WEEK=week of grazing season
DMYLDI=DM yield inside cage (kg ha^{-1})
DMYLDO=DM yield outside of cage (kg ha^{-1})
INTAKE=calculated forage consumed (kg ha^{-1})

APPENDIX 13 (Cont): Data on estimation of animal intake (DM basis) using samples taken in and out of exclusion cages in each paddock during grazing season. Study III.

VARIETY	PEN	WEEK	DMYLDI	DMYLDO	INTAKE
RIVAL	7	8	516.28	435.09	81.19
RIVAL	7	9	1116.58	1116.58	0.00
RIVAL	7	10	1155.92	1155.92	0.00
RIVAL	7	11	1157.89	898.14	259.75
RIVAL	7	12	990.03	1137.10	-147.07
RIVAL	7	13	905.39	882.94	22.45
RIVAL	7	14	1394.89	1135.44	259.45
RIVAL	7	15	1285.49	1286.13	-0.64
RIVAL	7	16	1249.56	1103.33	146.23
VENTU	4	1	957.91	957.91	0.00
VENTU	4	2	1677.48	1388.41	289.07
VENTU	4	3	2043.52	1911.15	132.37
VENTU	4	4	2718.99	902.46	1816.53
VENTU	4	5	561.16	518.68	42.48
VENTU	4	6	822.01	560.20	261.81
VENTU	4	7	641.65	633.44	8.21
VENTU	4	8	517.96	577.20	-59.24
VENTU	4	9	827.00	827.00	0.00
VENTU	4	10	1124.05	1124.05	0.00
VENTU	4	11	1290.44	1092.30	198.14
VENTU	4	12	1499.26	1118.13	381.13
VENTU	4	13	895.03	1173.57	-278.54
VENTU	4	14	1036.46	1340.45	-303.99
VENTU	4	15	953.26	953.26	0.00
VENTU	4	16	1525.80	1217.91	307.89
VENTU	6	1	507.90	507.90	0.00
VENTU	6	2	1178.02	985.51	192.51
VENTU	6	3	1338.59	1463.00	-124.41
VENTU	6	4	2000.52	989.15	1011.37
VENTU	6	5	1423.53	720.94	702.59
VENTU	6	6	667.49	634.11	33.38
VENTU	6	7	607.32	546.56	60.76
VENTU	6	8	638.31	480.35	157.96
VENTU	6	9	1104.73	1104.73	0.00
VENTU	6	10	900.84	900.84	0.00
VENTU	6	11	958.05	775.86	182.19
VENTU	6	12	1229.20	1057.71	171.49
VENTU	6	13	1495.45	1427.55	67.90
VENTU	6	14	1568.91	1436.31	132.60
VENTU	6	15	1270.41	1270.41	0.00
VENTU	6	16	1580.46	1308.88	271.58

VARIETY=cultivar PEN=paddock WEEK=week of grazing season
DMYLDI=DM yield inside cage (kg ha^{-1})
DMYLDO=DM yield outside of cage (kg ha^{-1})
INTAKE=calculated forage consumed (kg ha^{-1})

The values for DM yield inside and outside the cage are the means of samples taken from four exclusion cages in each paddock. This number of cages was not sufficient to give an accurate indication of the forage being consummed in the 0.8 ha paddocks. This was especially evident at times when only 10 lambs were grazing in each paddock.

During weeks 9 and 10 when lambs were not in their assigned paddocks, only one set of four samples were taken and used for both yield inside and outside of the cage.

APPENDIX 14: Individual data of lamb productivity while on finishing ration after removal from pasture. Study III.

VARIETY	SHEEP	INT	FIN	GAIN	ADG
c	54	31.6818	49.2727	17.5909	0.314123
c	73	35.5000	52.1364	16.6364	0.297078
c	153	25.6364	39.3636	13.7273	0.245130
c	180	33.0909	48.9773	15.8864	0.283685
c	184	33.3182	53.2727	19.9545	0.356331
c	223	36.0000	52.0455	16.0455	0.286526
c	248	34.0682	53.8182	19.7500	0.352679
f	31	34.1136	48.5000	14.3864	0.256899
f	113	24.2500	43.4091	19.1591	0.342127
f	152	30.7727	44.2727	13.5000	0.241071
f	164	22.5455	43.0455	20.5000	0.366071
f	233	26.8864	44.3409	17.4545	0.311688
f	235	30.7727	47.9545	17.1818	0.306818
f	260	28.5227	46.6364	18.1136	0.323458
r	7	33.9545	52.2955	18.3409	0.327516
r	66	31.5909	55.9773	24.3864	0.435471
r	126	31.3182	48.6818	17.3636	0.310065
r	185	35.5227	53.5227	18.0000	0.321429
r	239	24.7727	43.4545	18.6818	0.333604
r	249	25.1136	39.3409	14.2273	0.254058
r	253	27.0909	41.9091	14.8182	0.264610
v	55	29.5455	43.0909	13.5455	0.241883
v	111	26.8636	46.9773	20.1136	0.359172
v	167	39.8636	58.4318	18.5682	0.331575
v	181	24.0909	42.4091	18.3182	0.327110
v	205	34.2273	50.0909	15.8636	0.283279
v	259	22.3864	41.6136	19.2273	0.343344

VARIETY=cultivar lambs were grazing prior to trial PEN=paddock
INT1=initial weight (kg) FIN1=final weight (kg)
TOTAL1=weight gain (kg) ADG1=ADG (kg day^{-1}) c=Champ f=Frontier
r=Rival v=Venture

APPENDIX 15: Analysis of variance for alkaloid and nutrient contents of RCG samples collected during period I of the grazing trial. Study III.

Parameter	Source	df	Anova SS	F value	Pr > F
Gramine	cultivar	2	17.1996	1.59	0.3375
	paddock(cultivar)	3	16.1833	5.16	0.0095
	week	6	117.5622	18.75	0.0001
	cultivar*week	12	12.0778	0.96	0.5139
	error	18	18.8141		
Hordenine	cultivar	2	6.7809	2.09	0.2701
	paddock(cultivar)	3	4.8663	4.72	0.0133
	week	6	17.9002	8.69	0.0002
	cultivar*week	12	2.0229	0.49	0.8942
	error	18	6.1797		
Crude protein	cultivar	2	14.9295	1.08	0.4433
	paddock(cultivar)	3	20.7341	2.58	0.0896
	week	6	732.2593	45.48	0.0001
	cultivar*week	12	21.5001	0.67	0.7599
	error	18	48.3021		
Acid detergent fiber	cultivar	2	26.5030	1.19	0.4158
	paddock(cultivar)	3	33.3327	2.21	0.1223
	week	6	541.3000	17.93	0.0001
	cultivar*week	12	58.7893	0.97	0.5057
	error	18	90.5659		
Neutral detergent fiber	cultivar	2	35.6241	3.91	0.1461
	paddock(cultivar)	3	13.6714	1.90	0.1651
	week	6	500.2002	34.84	0.0001
	cultivar*week	12	49.4397	1.72	0.1442
	error	18	43.0710		

APPENDIX 16: Analysis of variance for mineral contents of RCG samples collected during period I of the grazing trial. Study III.

Parameter	Source	df	Anova SS	F value	Pr > F
Calcium	cultivar	2	0.008355	0.93	0.4852
	paddock(cultivar)	3	0.013485	6.54	0.0255
	week	2	0.050820	37.00	0.0004
	cultivar*week	4	0.002825	1.03	0.4636
	error	6	0.004121		
Phosphorus	cultivar	2	0.004647	1.10	0.4387
	paddock(cultivar)	3	0.006348	2.96	0.1194
	week	2	0.106218	74.36	0.0001
	cultivar*week	4	0.004074	1.43	0.3317
	error	6	0.004285		
Magnesium	cultivar	2	0.002541	2.33	0.2453
	paddock(cultivar)	3	0.001702	0.45	0.7237
	week	2	0.033670	13.48	0.0060
	cultivar*week	4	0.004187	0.84	0.5478
	error	6	0.049677		
Copper	cultivar	2	0.340759	1.55	0.3452
	paddock(cultivar)	3	0.330208	0.12	0.9457
	week	2	116.346445	62.86	0.0001
	cultivar*week	4	2.976175	0.80	0.5648
	error	6	5.552336		
Zinc	cultivar	2	97.746474	2.86	0.2017
	paddock(cultivar)	3	51.231087	1.71	0.2644
	week	2	198.886537	9.93	0.0125
	cultivar*week	4	23.673987	0.59	0.6821
	error	6	60.074064		
Sulfur	cultivar	2	0.662710	2.15	0.2637
	paddock(cultivar)	3	0.462863	1.16	0.3993
	week	2	0.196788	0.74	0.5162
	cultivar*week	4	0.348997	0.66	0.6441
	error	6	0.797894		

APPENDIX 17: Analysis of variance for alkaloid and nutrient contents of RCG samples collected during period II of the grazing trial. Study III.

Parameter	Source	df	Anova SS	F value	Pr > F
Gramine	cultivar	2	18.6524	95.81	0.0019
	paddock(cultivar)	3	0.2920	0.34	0.7999
	week	6	17.3672	9.97	0.0001
	cultivar*week	12	7.3783	2.12	0.0729
	error	18	5.2249		
Hordenine	cultivar	2	6.8289	8.87	0.0550
	paddock(cultivar)	3	1.1549	2.13	0.1319
	week	6	8.8539	8.17	0.0002
	cultivar*week	12	1.6058	0.74	0.6978
	error	18	3.2518		
Crude protein	cultivar	2	13.0339	2.59	0.2218
	paddock(cultivar)	3	7.5378	1.64	0.2154
	week	6	317.5494	34.55	0.0001
	cultivar*week	12	14.9125	0.81	0.6375
	error	18	27.5751		
Acid detergent fiber	cultivar	2	31.7258	10.81	0.0426
	paddock(cultivar)	3	4.4023	0.59	0.6298
	week	6	61.1064	4.09	0.0092
	cultivar*week	12	28.2023	0.94	0.5290
	error	18	44.8331		
Neutral detergent fiber	cultivar	2	50.9859	15.69	0.0258
	paddock(cultivar)	3	4.8747	0.99	0.4196
	week	6	74.0539	7.52	0.0004
	cultivar*week	12	36.5224	1.86	0.1144
	error	18	29.5292		

APPENDIX 18: Analysis of variance for mineral contents of RCG samples collected during period II of the grazing trial. Study III.

Parameter	Source	df	Anova SS	F value	Pr > F
Calcium	cultivar	2	0.003975	0.33	0.7413
	paddock(cultivar)	3	0.001800	3.72	0.0803
	week	2	0.009768	3.03	0.1234
	cultivar*week	4	0.005288	0.82	0.5573
	error	6	0.009684		
Phosphorus	cultivar	2	0.010592	1.53	0.3490
	paddock(cultivar)	3	0.010412	8.15	0.0154
	week	2	0.028104	33.01	0.0006
	cultivar*week	4	0.001557	0.91	0.5123
	error	6	0.002554		
Magnesium	cultivar	2	0.001062	0.18	0.8461
	paddock(cultivar)	3	0.009004	1.38	0.3354
	week	2	0.011315	2.61	0.1526
	cultivar*week	4	0.007502	0.86	0.5353
	error	6	0.013017		
Copper	cultivar	2	2.199925	0.84	0.5128
	paddock(cultivar)-	3	3.921712	1.73	0.2607
	week	2	11.569890	7.63	0.0224
	cultivar*week	4	4.278269	1.41	0.3356
	error	6	4.546422		
Zinc	cultivar	2	170.324607	4.48	0.1257
	paddock(cultivar)	3	57.074321	6.81	0.0233
	week	2	183.678259	32.86	0.0006
	cultivar*week	4	67.974741	6.08	0.0264
	error	6	16.769938		
Sulfur	cultivar	2	0.024372	1.15	0.4263
	paddock(cultivar)	3	0.316625	0.58	0.6484
	week	2	0.010929	0.03	0.9705
	cultivar*week	4	0.333120	0.46	0.7645
	error	6	1.088656		

APPENDIX 19: Analysis of variance of weight gains for lambs during period I and period II of grazing trial. Study III.

Source	df	Type III SS	F value	Pr > F
Cultivar	2	23628	7.19	0.0717
Paddock(cultivar)	3	4298		
Period	1	8869	6.37	0.0859
Cultivar*period	2	23634	8.48	0.0582
Period*paddock(cultivar)	3	4179		
Error	98			

APPENDIX 20: Analysis of variance of weight gains for lambs while on finishing ration after removal from grazing trial. Study III.

Parameter	Source	df	Type III SS	F value	Pr > F
Initial weight	cultivar	3	75.3564	1.16	0.3448
	error	23	496.2106		
Final weight	cultivar	3	69.5128	0.85	0.4825
	error	23	629.3591		
Weight gain	cultivar	3	3.4939	0.16	0.9192
	error	23	162.8167		
Average daily gain	cultivar	3	0.001114	0.16	0.9192
	error	23	0.051918		

APPENDIX 21: Coefficients used for non-linear regressional analysis for changes in alkaloid and nutritional components during grazing season. Study III.

	A2	B1	B2	C1	C2	week0
Frontier						
paddock 1						
gramine	0.0970	-0.8774	0.5322	0.2234	-0.0302	5.0605
hordenine	0.0970	0.5188	0.7423	-0.0348	-0.0405	6.5033
CP	9.1854	7.3652	2.5645	-2.3866	-0.1522	3.8308
ADF	30.5325	-17.1725	0.3368	4.3801	-0.0106	3.2273
NDF	60.1569	-7.1843	0.4541	2.4813	-0.0229	3.0625
paddock 2						
gramine	5.2474	-1.6428	-0.3151	0.3194	0.0080	6.0000
hordenine	2.8269	0.0033	0.0703	0.0342	-0.0063	6.0000
CP	-7.2197	0.5724	4.7238	-0.6949	-0.2211	5.3873
ADF	45.6178	-4.6559	-1.8527	1.2141	0.0762	5.0516
NDF	58.5477	-3.2998	1.5232	1.4565	-0.0906	3.4136
Rival						
paddock 1						
gramine	17.6505	-2.5107	-1.7000	0.5869	0.0471	6.0814
hordenine	6.0573	1.1471	-0.1325	-0.0512	-0.0050	6.0000
CP	5.2541	1.3152	3.2023	-0.8803	-0.1811	4.5724
ADF	39.8616	-4.9835	-1.1607	1.2899	0.0516	4.8148
NDF	65.6009	-1.0711	-0.3281	-0.4997	0.0058	5.1689
paddock 2						
gramine	-4.1775	0.7191	1.6949	0.1926	-0.0804	3.5023
hordenine	0.6942	1.1397	0.6118	-0.1064	-0.0331	5.5039
CP	0.9922	5.0388	3.8243	-1.2990	-0.2079	4.9897
ADF	35.3487	-0.6052	0.0561	0.2417	-0.0127	7.0000
NDF	69.1238	-3.6624	-0.6632	1.0247	0.0147	5.0000
Venture						
paddock 1						
gramine	3.8961	-1.7767	0.0413	0.2178	-0.0130	6.5947
hordenine	2.7046	0.8253	0.0500	-0.0836	-0.0083	5.1542
CP	-1.3117	0.0659	3.9848	-0.9029	-0.2044	4.4926
ADF	51.6932	-5.2844	-3.1442	1.7754	0.1454	4.4030
NDF	71.6771	-10.2125	-1.3684	3.2183	0.0609	3.5744
paddock 2						
gramine	4.3875	-2.2794	-0.0493	0.3770	-0.0080	6.0891
hordenine	0.3688	-3.5205	0.6414	0.8698	-0.0367	3.1632
CP	0.9446	0.2602	3.6128	-0.5950	-0.1972	5.1001
ADF	39.9311	-1.5680	-1.2763	0.6079	0.0692	5.0000
NDF	73.0377	-5.0807	-1.5372	1.4233	0.0692	4.3666

APPENDIX 21 (cont): The regression equation used to describe changes in gramine, hordenine, CP, ADF and NDF contents during the grazing season in 1988 was composed of two quadratic equations.

If week < week0, then the equation was;

$$\text{content} = A1 + B1*\text{week} + C1*\text{week}^2$$

$$\text{where } A1 = -(B1*\text{week0} + C1*\text{week0}^2) + \text{intercept}$$

$$\text{where the intercept} = A2 + B2*\text{week0} + C2*\text{week0}^2$$

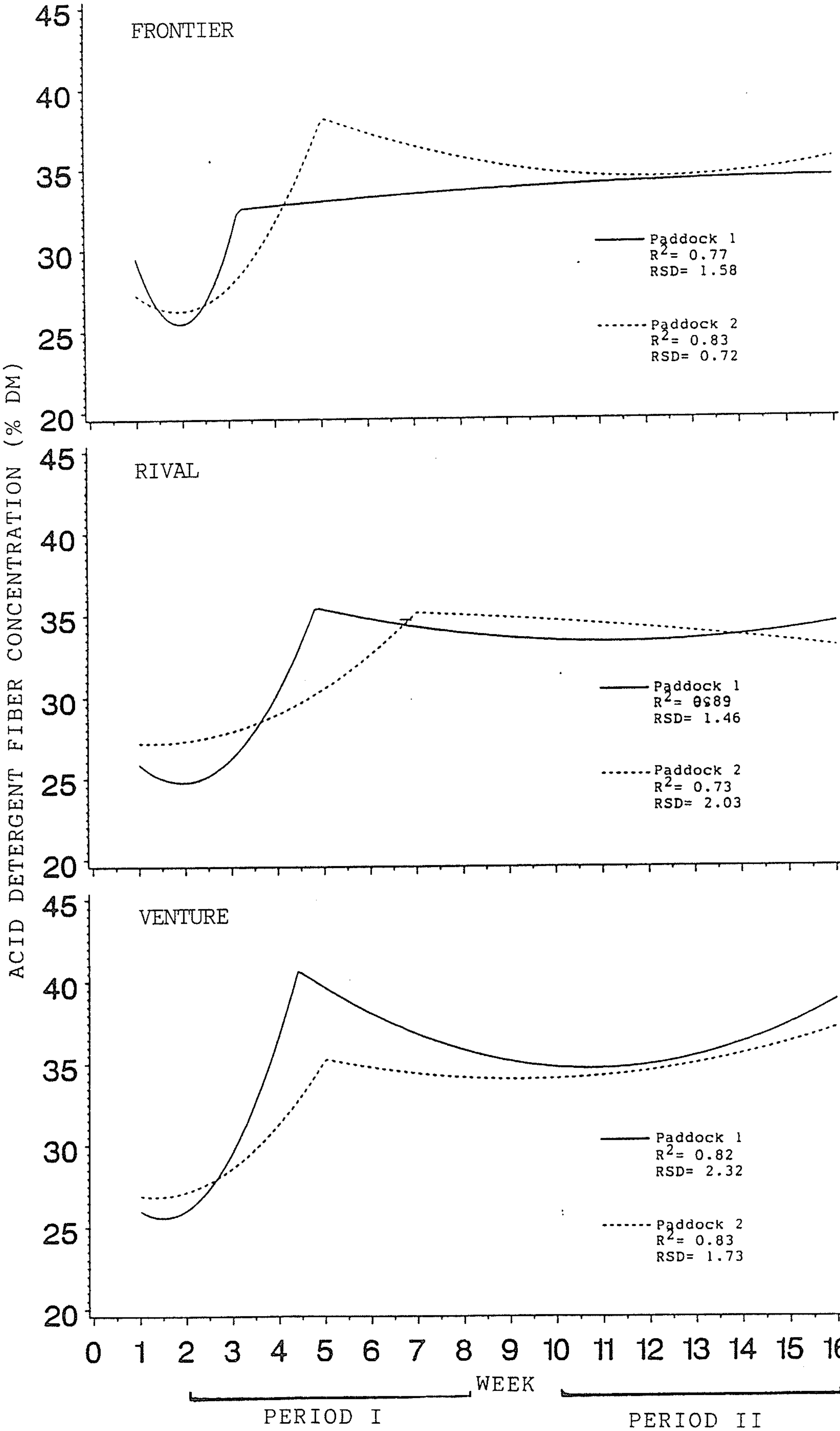
if week > week0, then the equation was;

$$\text{content} = A2 + B2*\text{week} + C2*\text{week}^2$$

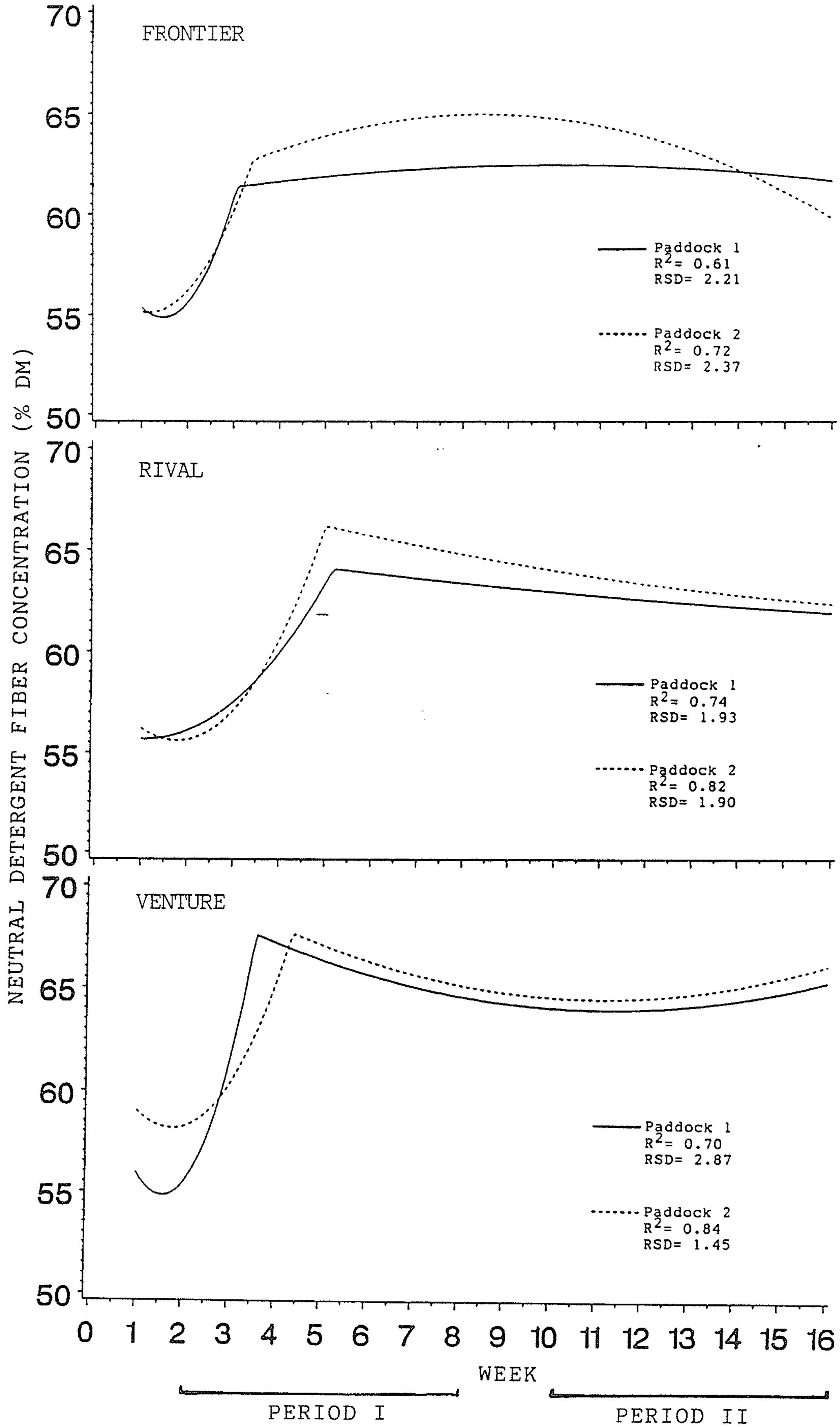
where week=period of time during the grazing season and
week0=period in time where the two quadratic equations meet.

Equations were generated with non-linear regressional analysis (SAS 1985).

APPENDIX 22: Acid detergent fiber concentration (mg g^{-1} DM) of three cultivars of reed canarygrass during summer grazing 1988. Concentrations estimated with the use of nonlinear regression equations. Study III.


FRONTIER
RIVAL
VENTURE
ACID DETERGENT FIBER CONCENTRATION (% DM)
45
40
35
30
25
20
Paddock 1
R^2= 0.77
RSD= 1.58
Paddock 2
R^2= 0.83
RSD= 0.72
Paddock 1
RSD= 1.46
Paddock 2
R^2= 0.73
RSD= 2.03
Paddock 1
R^2= 0.82
RSD= 2.32
Paddock 2
R^2= 0.83
RSD= 1.73
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEK
PERIOD I
PERIOD II

APPENDIX 23: Neutral detergent fiber concentration (mg g^{-1} DM) of three cultivars of reed canarygrass during summer grazing 1988. Concentrations estimated with the use of nonlinear regression equations. Study III.


NEUTRAL DETERGENT FIBER CONCENTRATION (% DM)
FRONTIER
Paddock 1
R2= 0.61
RSD= 2.21
Paddock 2
R2= 0.72
RSD= 2.37
RIVAL
Paddock 1
R2= 0.74
RSD= 1.93
Paddock 2
R2= 0.82
RSD= 1.90
VENTURE
Paddock 1
R2= 0.70
RSD= 2.87
Paddock 2
R2= 0.84
RSD= 1.45
WEEK
PERIOD I
PERIOD II