Monitoring pasture quality using brix measurements

Novel Ways, Hamilton
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17/12/08

What is a brix measurement?

A refractometer is one of many tools which can be used to monitor pasture quality. It uses the known refractive index of a glass prism to measure the refractive index of a liquid, normally the fruit or pasture sap. This is done by either passing light through the sap, or reflecting light off the sap surface, and measuring the angle of the light. A brix meter refractometer is calibrated to give a percentage value of the dissolved sucrose to water ratio in a solution, relative to 20°C. This is called a Brix measurement. However, for example, a brix measurement of 25% means there is 25 grams of soluble content and 75 grams of water in 100 grams of solution. A measurement will be affected by all soluble compounds in a solution, as they all have an affect on the refractive index. Brix measurements are extensively used in the fruit and vegetable industry to monitor the quality of produce, for import/export standards and consumer quality checks. They are also used in the wine making industry, where the brix meter is an invaluable tool used to decide when grapes should be harvested.

There are two types of refractometers, optical and digital. Optical versions do not use any electronics, instead using daylight passed through a glass prism. The reading is read through an eyepiece, and the refracted light angle is measured on an optical scale by the user. Digital refractometers use a LED to reflect light off the prism/sap interface and a small digital sensor is used to calculate the angle of the reflected light. Digital refractometers have the advantage that they automatically correct for temperature variation. Both are the same in terms of precision (the most precise measurement it can make).

Forages are composed of many soluble and non-soluble compounds. Water soluble compounds (WSC) include sucrose, fructans, minerals, proteins, lipids, pectins and acids. A refractometer can be used to take a brix measurement of these soluble compounds in a forage sample. This gives a value of the soluble content in a grass sample, and multiple samples taken throughout a paddock will give an estimate of the average WSC content in the pasture. This should allow effective monitoring of changing pasture sugar content and corrective methods can be employed if the sugar content is low. However this assumes a major affect on refractive index comes from the WSC's compared with other soluble content in a forage sample.

A pasture sample is usually done by picking a variety of forages similar to what a ruminant would eat. This grass is squeezed onto the refractometer surface using a garlic press. The sample can be rolled prior to pressing the grass to "loosen" the content of the sample. A brix value can then be obtained from the optical or digital refractometer. However there are many variables which can affect a brix reading and these are examined later in this report.

Why have high sugar content pastures?

Ruminant animals are relativity inefficient at converting grass proteins to milk proteins, only achieving approximately 20% to 25% conversion efficiency. On top of this, some proteins are not well utilized by the animal. The total milk output of a cow can be increased by either improving this conversion efficiency or increasing the total grass intake of the cow. Research proves there is some correlation between this conversion efficiency and high sugar content on a farm. IGER Innovations produced research in 2001 suggesting high sugar grasses have a positive effect on the efficiency of milk production in an animal.

Grass is broken down in the rumen of a cow, producing amino acids to grow and produce more protein which is later used for milk production by the cow. However when the diet lacks readily available energy such as sugars, rumen microbes either cannot grow or, instead use amino acids to provide energy, meaning less milk production. Feeding energy-rich foods in a concentrate feed is one way to increase the efficiency of the rumen, however the cheaper way is to use the sugars which naturally occur in forages, (Moorby, 2001).

Many research papers show nitrogen fertilisation will directly increase the growth rate of a pasture; in particular Moller (1996) shows this effect. However, nitrogen application also significantly depresses the soluble carbohydrate levels, and it was suggested because of this, weight gain or milk production would be negatively affected (Moller, 1996).

One study produced by IGER Innovations, shows an 8% increase in milk production from cows which were grazed on high sugar ryegrasses, although they also had higher dry matter intakes (Miller et al, 1999). It was suggested that higher sugar grasses increase animal performance, and also increase feed nitrogen utilization, and reduce nitrogen excretion. Research has also been done which proves live-weight gains of other animals can be improved by using high sugar grasses (Downing & Gamroth, 2007).

From this research done at IGER, AberDart and other varieties of high sugar grasses were developed and released. This has lead to various reports and articles of farmers using high sugar grasses and obtaining a noticeable increase in productivity. A Hawkes Bay farmer stated increases from 6900 litres p/day to on average 7250 litres p/day, when the herd was shifted from normal grass to AberDart grass, (Straight Furrow, 2008). AberDart grass was also sown by a UK farmer in early January 2007. It was stated that the milk yield per cow increased from 22 to 25 litres per day. It is also stated the farmer found these grasses more resistant to drought, and recovered well after a dry spell, (MacKenzie, 2007).

In the summer 2007 Edition of NZ Grassland News, high sugar grasses were a feature of the modern developments in pasture science. It was stated that, from their trials, that milk yields from high sugar pastures were significantly higher than normal pasture grasses. It is suggested there is not a lot more research to be done before the theory becomes widely accepted by NZ farmers, (Allison, 2007).

A more widely accepted practice is to grow high sugar content grasses for silage and hay production. Cutting silage and hay when it contains higher sugar content means more sugar is retained in the feed and this will be present when it is fed to an animal. These high sugar levels allow the animal to digest it more efficiently, as fermentation in the rumen is more efficient. Quality silage and hay generally should have high sugar content, low nitrate levels and high digestibility values, so sugar content is one of a few important factors affecting feed quality.

From this literature research we can conclude that higher sugar content in forage tends to increase the efficiency in a ruminant animal. Research also suggests there may be some correlation between high sugar content in grass and higher dairy milk production per kilogram of dry matter, and further tests should be done to test this. The brix of milk can also be tested, indicating the quality of the milk. If the method is proven, higher brix measurements of milk may equate to more dollars per kg of dairy milk.

What is physically happening when I take a brix measurement?

Light is reflected off the grass sap and the critical angle at which this light stops reflecting is dependant on the sap composition that we are measuring. Therefore knowing exactly what is in grass sap, and how light interacts with this sap is important. This will allow us to know exactly what our brix measurement means when measuring grass.

When we talk about light reflecting off a surface, we treat the water soluble content as a whole and the fibrous content as individual molecules scattered through the water soluble content. This makes it easier to understand how the light will be reflected off the surface. Light hitting a water soluble area will reflect/refract, according to the refractive index of the whole of the water soluble sap content. Light hitting fibrous compounds will most likely be scattered or absorbed. This is due to the definition of refractive index (RI), which is directly related to the density of the material each photon hits.

$$RI = c / c_m \qquad \text{Equation 1.}$$

Where c = speed of light in vacuum
c_m = speed of light in material

Therefore, the more dense the compounds in the sap, the slower the light travels through the sap, and the higher the refractive index (by equation 1). Localised areas such as fibrous compounds will have an extremely low refractive index and will not contribute to the reflected light, and hence the brix reading.

Since the water soluble content of the grass sap will contain many compounds which affect the refractive index differently, we must now consider what exactly is in the sap after it is squeezed from the garlic press. Grass uses the process of photosynthesis to produce sugar. This sugar is namely glucose straight after photosynthesis, but is quickly turned into sucrose or fructans. Sucrose is used to transport the glucose and fructose around the plant for use or storage as fructans and starch, and is found throughout the grass structure. Fructans are one sucrose molecule attached to a chain of fructose, and are mainly found in the leaves of the grass. Starch is mainly stored in the root of the plant for later use.

When cutting a piece of grass, the cell walls, made up of cellulose, hemicellulose, glucans and pectins, are broken up and introduced to the sample. In small concentrations this will not affect the brix reading because the majority of the sap/prism interface does not contain fibrous compounds. However if a grass sample is ground and measured, the sap/prism interface would be saturated with fibrous compounds, and hence the reflected light in the refractometer would not be representative of the water soluble content. Effectively we would measure the refractive index of fibre, not sugar content. By simply crushing the sap out of grass, it is not expected that much fibrous content would be introduced to the sample, as the cell walls stay intact, and are not physically broken or ripped apart.

What affects the accuracy of a pasture brix measurement?

Results as of 17/11/08

Brix measurements taken in the field with a refractometer can be exceedingly dependent on different factors. Ambient temperature, sample preparation, sample settling time and sample location affect the brix readings. Also the type, maturity and segment of grass sampled, the time of year and the time of day will change a brix reading. These variables must be considered in order to form a generalised method of grass sampling. This should allow a more robust and standardised process, which can be used by all farmers.

Firstly, research was done to assess exactly what compounds in plant sap will affect the internal reflection angle, and consequently the brix reading the device gives. Wilson (1995) shows the percentage dry matter composition of perennial rye grass and white clover below in Table 1. One notable point is NSC (Non-Structural Carbohydrates) which is largely made up of sucrose and fructans.

Table 1, Composition (% Dry Matter) of White Clover and Perennial Ryegrass (Wilson, 1996)

	White Clover	Perennial Ryegrass
Protein	25.9	20.5
Hemicellulose	9.7	12.6
Cellulose	20.9	25.4
Lignin	8.2	6.8
Ash	12.7	10.3
Assumed Fat	4	6
NSC	18.6	18.4
Digestibilty (%)	76.5	74.3

The maturity of a grass plant will affect its composition and hence the brix reading from a refractometer. Wilson (1995) also shows generally how pasture composition changes with maturity. Figure 1 shows the major grass content and how it changes over time (all values are generalised). It shows that grass sugar percentage is related to the maturity and hence the harvesting stage of the plant.

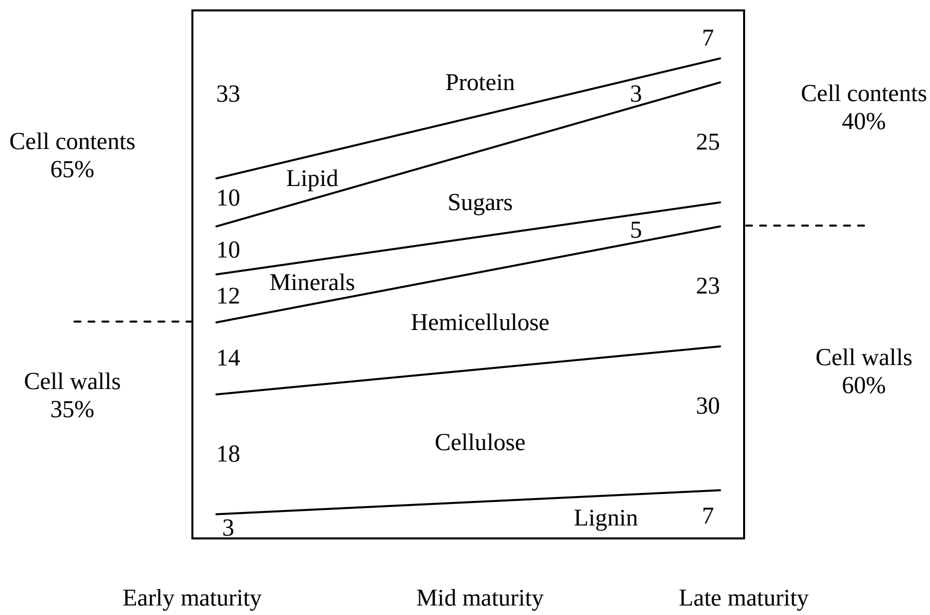

Figure 1, Effects of stage of maturity on pasture composition, during Spring. (Wilson, 1995)

Ambient temperature physically affects the refractive indices of the mediums used in the refractometer. As temperature increases, the refractive indices of the refractometer prism and the grass sample decrease, introducing inaccuracy to a brix measurement if there is a large temperature difference between the sample and the prism. This error is compensated for in digital refractometers, however approximately 40 to 60 seconds must be allowed in each measurement to let this compensation work. In order to illustrate this, a simple temperature test was done to show a sample changing over time, where the results are seen in Figure 1, done using an Atago PAL-1 digital refractometer.

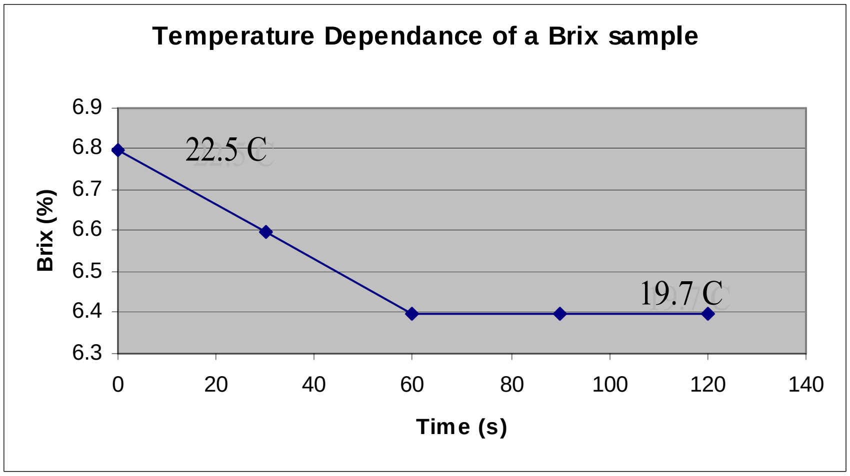

Figure 1, Temperature dependence of a brix reading.

It was then found that a brix sample will also decrease under stable temperature conditions. Figure 2 shows this change over 120 seconds. The main process behind this is thought to be enzymatic changes happening after the sap has been squeezed onto the refractometer.

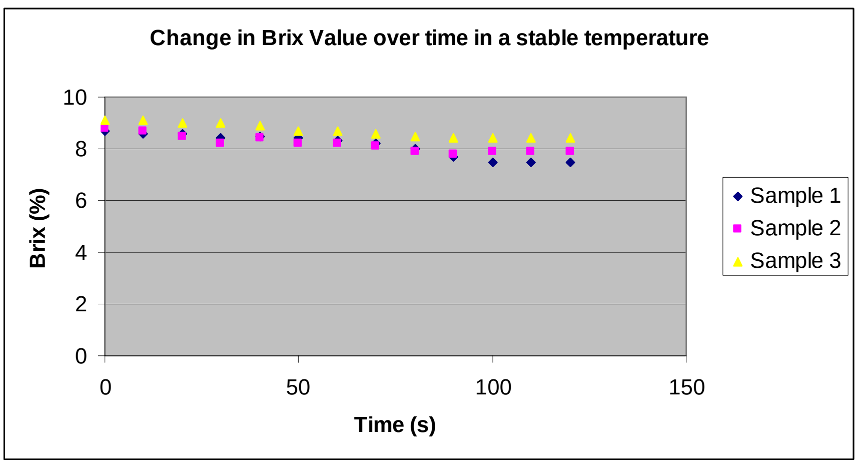

Figure 2, Change in Brix value over time in a stable temperature

Sample preparation has a large effect on the brix reading of a grass sample. Tests were done showing that a grass sample brix measurement can vary from ~2.0% to 10.3% depending on how long the sample is crushed and rolled between two hands. This is possible because more structural carbohydrates are introduced to the sample sap, therefore increasing the refractive index of the sap, or that the sap is broken out of the plant in a more representative way, reflecting the true WSC percentage. A simple rolling experiment was done multiple times to show how this changes the brix reading, and this is shown in Figure 3 using the time the sample is rolled/crushed versus the brix reading. It is also recommended that the actual grass sample taken is the same part of plant a ruminant animal would ingest. This is approximately 5 cm above the dirt for cows.

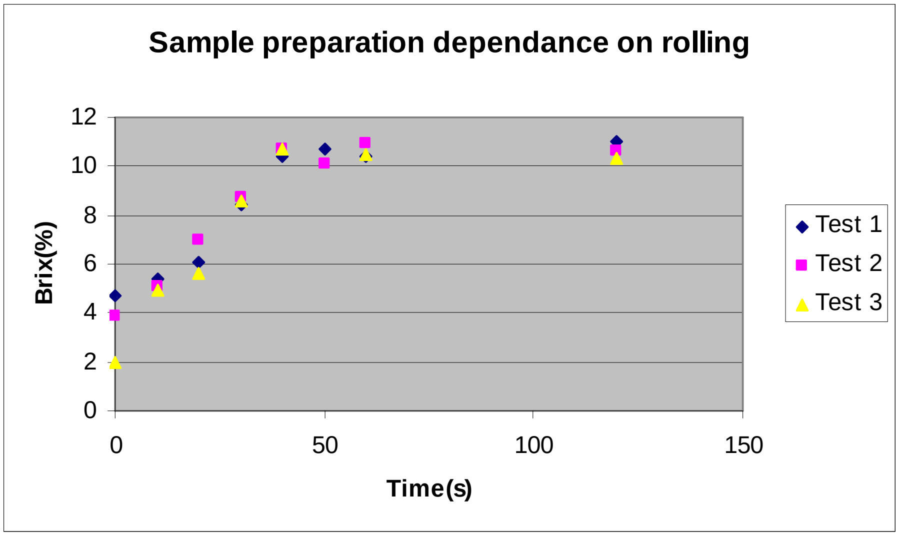

Figure 3, Rolling dependence of a brix reading.

Readings should be taken during the same time of day, and under the same weather conditions leading up to the measurement in order to get any form of comparative sampling with your grass. This is because all these factors affect the sucrose level in the actual grass plant. Downing and Gamroth (2007) produced a quality experiment showing these effects on the soluble content in grass or the NSC over a one year period. This was done for multiple grass species with average readings calculated for a whole day. This is seen below in Table 2 (Downing and Gamroth, 2007).

Table 2, Nonstructural carbohydrate % of dry matter for am/pm harvested cultivars

	Percent total nonstructural carbohydrate in dry matter						
	4/20 a.m.	4/20 p.m.	6/28 a.m.	6/28 p.m.	10/1 a.m.	10/1 p.m.	Total
Elgon	14.3	13.7	18.2	25.3	21.0	23.9	19.4
Tetralite	14.8	14.7	16.3	25.1	19.4	23.9	19.0
Herbie	11.5	13.3	19.2	25.9	19.6	21.7	18.5
BG-34	12.6	16.2	17.2	19.4	17.1	27.3	18.3
Tonga	15.8	21.7	16.0	20.6	15.2	20.0	18.2
Glenn	13.8	17.6	17.0	19.1	19.5	21.8	18.1
Bison	12.1	12.4	18.4	24.5	15.9	22.9	17.7
Matua	12.2	22.6	14.5	21.9	15.5	18.7	17.6
Barfort	14.4	18.0	14.0	23.5	13.8	20.3	17.3
Flanker	12.9	13.6	17.7	21.0	15.2	23.5	17.3
Belramo	12.5	14.1	14.6	19.2	18.3	17.5	16.0
Bronsyn	9.0	15.1	17.6	21.2	13.0	16.5	15.4
Barfest	11.7	15.9	13.5	12.2	13.3	21.8	14.7
Orion	14.3	11.9	17.1	22.4	10.2	11.1	14.5
Pizza	10.0	12.4	12.3	19.6	8.2	12.5	12.5
Cambria	9.7	14.8	9.1	15.6	10.3	12.8	12.0
Baridana	10.8	11.6	8.8	14.0	9.0	16.5	11.8

(Note: 4/20 is Mid Spring, 6/28 is Early Summer, 10/1 is Early Winter.)

Each grass species is quite variable in terms of their NSC content readings. However the data shows there is a solid trend between seasonal and daytime readings. The NSC content in a grass sample will reach its peak in the afternoon. The NSC content in a grass sample will be larger in the warmer months of the year and smaller in the cooler months of the year. This must be considered when taking brix measurements.

Obviously different areas of a farm paddock will produce different brix measurements because of the natural layout, pasture composition, uneven fertilisation and animal excretion patches. This error can be overcome by taking multiple samples throughout a paddock. Once a stable reading is reached, this can be taken as the average brix for the paddock. To give an idea of which grasses are have higher brix values, samples were taken using common grass types in NZ pastures. These results are shown in Table 3, done without rolling or preparing the sample, and at the same time of day. Obviously these values will vary for different farms, but it gives an indication of the difference between grass types.

Table 3, Comparison of common NZ pasture grasses.

Types of grass	Brix range (comparative)
Ryegrass	4.5 - 4.7
Cocksfoot	2.0 - 2.5
Orchard Grass	2.0 - 2.5
Legumes	3.0 - 4.3
Plantains	3.5 - 4.5
Other	4.0 - 4.5

This suggests targeting one species, like ryegrass, and only one part of the grass, like blades, may give more accurate reading. This accuracy comes from less error being introduced by the difference in species, and sugars being stored at different locations in the plant structure.

Care must be taken to make sure the sample does not have excess water and dirt on it, as water especially will influence the brix reading. Measurements should not be taken in wet conditions, and in damp conditions the grass should be dried using a paper towel. If the sample grass has excess dirt, the grass sap can be filtered using standard chemical filter paper. However for best results the sample should avoid both dirt and excess water.

Barometric pressure will also affect the WSC in a grass sample. This is because a plant can sense changes in barometric pressure, as a warning of an impending storm. The plant then starts to store carbohydrates in its root system, so that if the conditions worsen, it may have a better chance of surviving. Therefore any samples done prior to bad weather may effect brix measurements.

Optical refractometers may be slightly less accurate than digital refractometers due to human error. This is simply a function of the user having to make judgment on where the shadow line falls on the optical scale. Using a digital refractometer means this shadow line point is chosen at exactly the same point, meaning less variation between successive readings.

How do I take accurate brix measurements?

After doing multiple tests and consulting with farmers who have been using brix readings for years, a standardised way to sample grass using a brix meter is proposed.

1. Choose a clear sunny day, where bad weather is not expected for the next 24 hours or so. If unsure, check the barometric pressure making sure it is around 1013 hPa (NZ only).

2. According to your pasture composition, decide whether you would like to sample the paddock as a whole, or target an individual species such as ryegrass. The latter method should give more repeatability and accuracy.

3. Decide what part of the plant/s to sample, stem or leaves. Preference is to sample the blades of the plant.

4. Pick or cut the grass, according to how a ruminant would eat (approximately 5cm above ground level). If sampling the whole paddock, take many samples from around the paddock which are representative of the whole paddock. If using a selective sample, consider the urine and dung patches throughout a paddock. To get an accurate reading it is recommended to sample the whole paddock.

5. Roll the sample for approximately 60 seconds between your hands, or use a mechanical juicer. (Further tests may suggest a different method at this stage, for example no rolling of the sample).

6. Place sample in the garlic press, and squeeze three or four drops onto the refractometer well or optical plate. Consider temperature of the sample, and if there is a large difference between the sample and the refractometer well, wait for the sample to settle. However this is not recommended at small temperature differences as enzymatic changes will give greater error in the readings.

7. Take the immediate brix reading given by the refractometer, (assuming little temperature difference).

8. Take all grass brix measurements using exactly the same methods, at approximately the same time of day. This will greatly reduce the error between subsequent readings.

To understand the measurement you get from using this method, consider all the factors included in this report. These mainly include how you have prepared the sample, what stage of maturity the plant is at, and the history of the pasture (fertilisiser history, recent storms, droughts etc).

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Appendix A

SCOTT FARM TRIAL NOTES 20/11/08

Experiment

Introduction

An experiment was carried out on Scott Farm to find a correlation between pasture sugar content and brix measurements of the pasture using a digital refractometer. Grass samples were taken from 4 different paddocks, varying in grass variety and fertiliser input. The experiment was done over one day on the 21/11/2008, with two samples being taken at 11am, and 4 samples being taken between 2-4pm. Lab pasture analysis results were compared with brix readings.

Methodology

Two initial samples of a low nitrogen input paddock were taken at 11am. These included multiple brix measurements and a pasture sample of the whole paddock, and a selected square metre of the paddock. The pasture samples were refrigerated and sent to the lab later that day.

The same method was used later in the day four the same paddock at 2pm. Three more paddocks were sampled after that, only with a square metre pasture sample. All seven pasture samples were sent to Hills Laboratories for a pasture feed analysis which indicated soluble sugar content as a percentage of dry weight.

Three operators were used to reduce time between paddock samples. Each used a digital refractometer and took three brix readings. One grass sample tested un-rolled, one rolled for 10 seconds, and one rolled for 60 seconds.

Samples were taken by picking the grass similar to what a ruminant would eat, 5cm from the ground. This including picking a representative sample of all grass species in the paddock. They were then squeezed through a garlic press after their preparation method was complete.

Paddock Notes

C33 Tight nitrogen input : Mostly contains perennial ryegrass, with other varieties such as chicory, cocksfoot, weeds, and minimal clover. Contains approximately 5 worms per 8000cm^3 of soil. Noticeably greener than other paddocks because of nitrogen input. Varying urine and dung patches however not as noticeable as other paddocks. Grass nearing maturity.

C24a new ryegrass and chicory low nitrogen input : All Tetraploid ryegrass and chicory. Early maturity, No worms found in 8000cm^3. Quite patchy with noticeable difference between excretion areas. Ground is reasonably hard

C27a No Nitrogen : Rough paddock with many species of grasses and weeds. Ryegrass, plantains, chicory, clover, cocksfoot, etc. Had been grazed recently. Grasses are mature but short because of grazing. No Worms.

Crosby Farm: Recently been mowed for silage. Still showing dirt patches from drought last summer. Mainly ryegrass with some weeds such as thistle. Ground is hard, compact and dry.

Results

The soluble sugar content values obtained from the lab were plotted against the corresponding brix values obtained during paddock sampling. These soluble sugar values were corrected from % dry matter to % water of the grass sample, using the dry matter percentage values. This allowed the brix measurements (which are measured as % water) to be compared to the soluble sugar values. These correlations are seen in Figure A1, for not rolled, rolled for 10 seconds and rolled for 60 seconds samples. Rolled for 10 seconds shows the strongest correlation obtained.

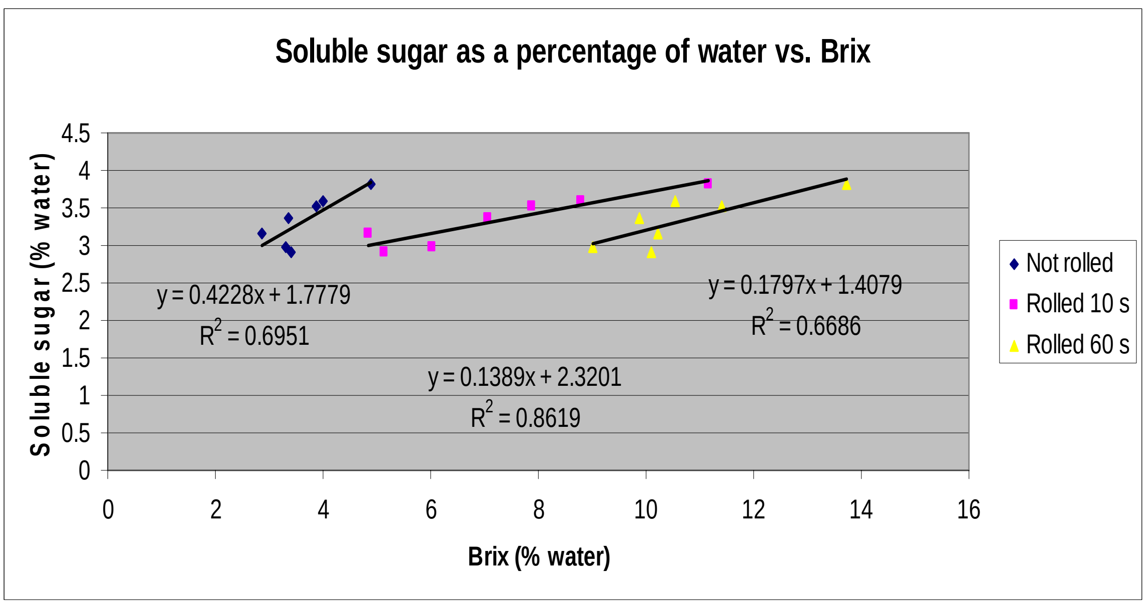

Figure A1, Brix vs. Soluble sugars in a pasture, sample rolled for 60 secs.

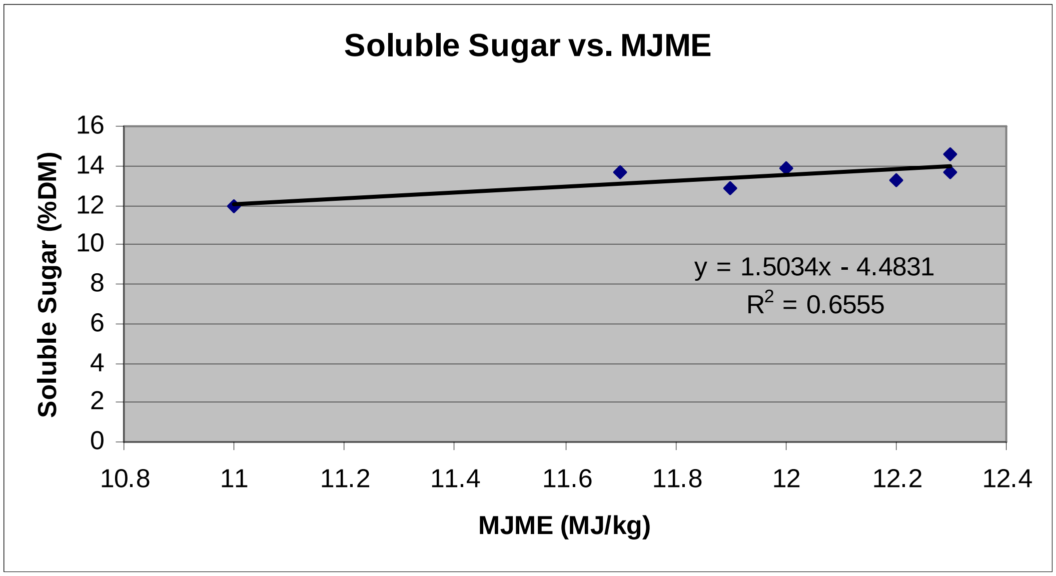

Figure A2, Soluble sugar vs. Metabolisable energy (MJME) for Scott farm trials

There is also an inherent correlation between the metabolisable energy and soluble sugar in the laboratory data. However this correlation is still weak as there are only seven points used by the data.

Table 1A, P values resulting from T Tests on sample data

T Tests	*Brix nr*	*Brix 10s*	*Brix 1min*
SS %(water)	0.245485987	0.000594875	3.03086E-08
SS %(water+DM)	0.002461792	0.00015347	9.94477E-09

All T tests done on the data, except for the Brix not rolled vs. soluble sugar (in % water), are well below the $P<0.05$ threshold. This means we can reject the null hypothesis quite confidently.

Discussion

The results suggest brix measurements give an indication of the actual sugar content in a pasture. Statistical analysis on the data show there is a high likelihood there is a relationship between soluble sugar and brix readings, as shown by the t tests. However, only seven data points have been used, and more added data points may change the correlation. These linear correlations work on the assumption that when sap is squeezed in a garlic press, as done in the brix sampling methods, the sugar levels are representative of the whole plant. Further plant sap analysis tests should prove that this assumption is justified.

The different sample preparation methods show a lot of variation between themselves. Because of this a relationship cannot be determined between the actual rolling methods. Further tests of lower brix pastures and possibly higher brix pastures can be added to the existing data, and could possibly improve the correlation. This is possible as long as the same sampling methods are done, and representative samples of the pasture are done (targeting all species in the paddock). As we are only looking at measured brix and real sugar content, new data can be added to the old data. This would allow a stronger relationship to be derived.

The strongest correlation was given when data from all three operators was used. Using data from selective operators significantly decreased any correlation even though various operators were expected to introduce some error into the results. This could be a result of many other effects, such as random sampling from the cut grass sample, or the variety of grass selected into each sample.

The correlation presented in figure A2 suggests brix measurements may also be able to estimate the metabolisable energy in the grass. This should also be proven when new data points are added to the existing data.

Conclusion

This initial experiment suggests there could be a strong correlation between brix pasture measurements and pasture sugar content. Secondary experiments should be carried out to add to the existing data to form a stronger correlation. Pasture sap from a garlic press should also be analysed to prove the assumption that the sap contains representative levels of the total sugar content.

Hills Laboratory Pasture Feed Analysis Results

Lab Results

sample name	sample	Dry Matter (%)	Crude Protein (%DM)	ADF (%DM)	NDF (%DM)	Ash (%DM)	DOMD	ME (MJ/kg)	Soluble Sugar
In C33 M	1	18	23.3	22.4	40.8	8.8	76.4	12.2	13.3
In C33 W	2	18.7	22.2	22.4	41.1	9.1	76.7	12.3	13.7
C33 M	3	19.4	22.3	21.6	39.7	8.3	76.6	12.3	14.6
C33 W	4	20.5	21.3	23.1	42.5	8.7	75.2	12	13.9
C27a M	5	22	15.4	26.9	48.2	9.6	68.5	11	11.9
C24a M	6	18.7	15.5	23.7	39.5	11.7	74.4	11.9	12.9
Crosby	7	21.8	20.7	23.4	42.8	10.6	73.2	11.7	13.7

Brix not rolled	Brix 10s	Brix 1min	Soluble Sugar %(water+DM)	Soluble Sugar %(water)
3.4	5.133333	10.1	2.394	2.9195122
2.866667	4.833333	10.23333	2.5619	3.15116851
3.866667	7.866667	11.4	2.8324	3.51414392
4	8.8	10.53333	2.8495	3.58427673
3.366667	7.066667	9.866667	2.618	3.35641026
3.3	6.033333	9	2.4123	2.96715867
4.9	11.16667	13.73333	2.9866	3.81918159

Brix Measurements of Pasture Samples

Not rolled	C27a M	C24a M	Crosby	C33 M	C33 W	In C33 M	In C33 W
1	4.5	2.9	5.5	3.3	3.7	4.3	3.3
2	2.6	3.6	4.2	4.3	3.7	3.4	3
3	3	3.4	5	4	4.6	2.5	2.3
average	3.366666667	3.3	4.9	3.866667	4	3.4	2.866667

10sec	C27a M	C24a M	Crosby	C33 M	C33 W	In C33 M	In C33 W
1	7.6	7.3	11.7	8.6	9.7	5.8	5.2
2	7.5	5.9	11	6.8	8.9	5.5	5.3
3	6.1	4.9	10.8	8.2	7.8	4.1	4
average	7.066666667	6.0333333	11.16666667	7.866667	8.8	5.133333	4.833333

1min	C27a M	C24a M	Crosby	C33 M	C33 W	In C33 M	In C33 W
1	11.6	9.1	14.2	11.7	10.3	10.4	10.8
2	9.8	8.9	13	11.6	11.7	9.8	9.7
3	8.2		14	10.9	9.6	10.1	10.2
average	9.866666667	9	13.73333333	11.4	10.53333	10.1	10.23333