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Individual feed intake and feed use efficiency in grazing animals

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Abstract

Pasture feed intake of individual animals is difficult to estimate. This project aimed to prove that a practical measurement system could open up the opportunity to select cattle for pasture feed use efficiency, which is an important economic trait. Ideas for a system were patented and a prototype system designed. Project objectives were to determine: 1) accuracy of the proposed system; 2) maximum number of head that could be serviced per bin and resulting test cost; and 3) repeatability of supplement and chaff intake level.

Cattle were fed chaff through autofeeders in a feedlot. Their recorded intakes of chaff were compared with that predicted by controlled feeding of wax labelled supplement from a purpose designed (Proway-Cottle-Dove) bin, followed by marker analysis of faecal and feed samples. Intake of native pasture was also estimated by the bin. Much technical development work was needed to tightly control individual supplement intake.

Autofeeder-recorded daily chaff intakes were variable and unreliable and so the results are of limited value. However, the correlations (r) between autofeeder and predicted lucerne chaff intake were 0.64-0.96 based on single faecal samples taken at 5-11 days. These correlations on lucerne:oaten chaff varied from 0.45- 0.69. The bin system used in this trial would estimate pasture intake at an approx. cost of $122/head. The repeatability of pasture feed intake across the year now needs to be determined.

Executive summary

Pasture feed intake by individual grazing cattle is very difficult to measure or estimate. Net/Residual feed intake (NFI/RFI) can be used to directly select for feed use efficiency and indirectly select for lower methane production. However, the high cost of RFI measurement in a feedlot (over $A500/head) and RFI's interaction with feed type and level (Herd et al. 2011) has limited its use by industry. Technically, the most successful approach to measure pasture intake has been to use indigestible, natural, plant markers, such as alkanes, recovered in faecal samples. However controlled release devices to deliver alkanes are no longer manufactured. Dr. Hugh Dove developed an alternate approach of feeding weighed amounts of wax-labeled supplement to dose animals. This has been turned into a more practical approach by enabling the animal to self-dose in the paddock via a purpose-built feed bin with EID tag reader, manufactured by Proway Livestock. The bin has mechanisms to control and record the daily labeled supplement intake of each animal. Bin purchasers will be provided with access to In-house algorithms that are used to calculate pasture intake estimations.

The objectives of this project were to prove the concept would work by: 1) determining the maximum number of head per bin and hence likely test cost, 2) determining the accuracy of the Proway-Cottle-Dove feed intake measurement system, and 3) estimating the daily repeatability of supplement and chaff intake in a feedlot environment. The approach used was to compare estimated chaff intakes from individual cattle fed labeled supplement from the prototype bin system with their actual chaff intakes recorded by auto-feeders in a feedlot. Pure lucerne chaff was fed *ad lib* in one trial run and a 50:50 lucerne:oaten chaff was fed *ad lib* in a second run. The bin was also used in a grazing situation to assess whether the pasture intake estimates were realistic based on cattle live weights.

The labelled supplement was palatable. The repeatability of daily chaff intake from auto-feeders in a feedlot was very low (e.g. 0.14 for lucerne chaff) due to competition for access to the autofeeder race and bin by cattle. The repeatability of daily pasture intake is probably much higher as animal competition for feed is much lower on pasture than in pen auto-feeders with single race entry.

The final prototype bin controlled the maximum daily supplement intake very well. The bin could service around 20 head of cattle. This will vary between groups of cattle. The approximate cost per animal tested, with a bin life of 20 years and 20 head per bin assumed and a marker test cost of $71.50/sample (revised NMI quote for a future commercial service) is $122/test, which is ~25% of the cost of a RFI test.

After studying many combinations of markers it was decided to use the data from six alkanes (C25, 27, 29, 30, 31, 33) and four alcohols (C24, 26, 28, 30) that occur most commonly in Australian grazed plant species. *The best algorithm for transforming and weighting marker concentrations will be kept commercial-in-confidence* and may be different from the discriminant weighting approach that was filed as a provisional patent in 2011 and is being prepared for publication in Anim. Sci. Feed Tech. (Cottle and Romero, in prep.). Further work is needed to finalise choice of transformation and weighting methods.

The actual intakes measured by the autofeeders in the feedlot were not reliable due to intermittent beam cutting problems and raw data transformation issues, so it was not possible to estimate the accuracy of the system. Rather the correlation of the two intake estimates are presented to demonstrate that intake predictions were in a feasible range, based on the liveweight of the cattle.

The correlation (r) between actual and predicted lucerne chaff intake based on a single faecal sample taken five days after labelled supplement intake was tightly controlled by the bins was 0.96 when data from one animal with more variable supplement intake was removed and the most effective algorithm was used. The correlation between predicted and actual was 0.79 when all animals with faecal samples were included. The correlations from faecal samples taken later in the lucerne chaff run were lower but acceptable, i.e. r= 0.64 for day 8 faeces and r=0.89 for day 11 faeces. This may have been due to the unexpected zero intakes of supplement on day 10 by three cattle that had very consistent supplement intakes from day 1 to day 9.

The correlation (r) between actual and predicted lucerne:oaten chaff intake based on a single faecal sample taken six days after animals had stabilised on the mixed chaff when data from one animal was removed was 0.69. The correlation between predicted and actual was 0.58 when all animals with faecal samples were included. The correlations from faecal samples taken later in the mixed chaff run were lower, i.e. r= 0.45 for day 9 faeces and r=0.62 for day 11 faeces. The reason for the lower correlation with mixed chaff is not known but continual, low repeatability (<0.03) of daily chaff intake, as measured by the autofeeders (Figure 14) made analyses difficult.

When the heifers grazed on a diverse species, improved native pasture, they had an average liveweight of 455kg in late April 2012. If total intake is estimated by the equation of Minson and McDonald (1987), then assuming a liveweight gain of 0.25kg/day in winter, the daily total dry matter intake of the heifers should have been about 11kg/day.

A principal component (PC) analysis of plant marker concentrations was carried out to establish *a priori* if the ten selected markers could distinguish between the plant species. PC scores 1 and 2 accounted for 98% of the variance in marker profile and their biplot showed that the labelled CSM and white clover would be easy to distinguish, whereas red grass, setaria and parramatta grass were difficult to distinguish from each other. This situation can often cause poor diet composition estimates and thus intake estimates. In this situation a marker profile for a single component called 'grass' can be calculated and the diet compositions can be estimated in terms of grass, white clover and CSM. This marker profile is best weighted for the proportions of the various grasses in the sward, however if these are unknown, their simple arithmetic mean can be used.

When the grasses were combined the diet composition, whole-diet digestibility and total daily intake of pasture plus pellet estimates, on average, accorded with the prediction of total intake from liveweight and gain. The average total intakes predicted from days 5, 7 and 10 faeces marker concentrations were 7.8kg, 6.9kg and 9.7kg/head respectively. There was a high between animal variation in predicted pasture intakes. This analysis confirms that in pastures with many species it may be necessary to group grasses together before proceeding to weighted least squares analyses to estimate pasture intake.

The average daily intake of pasture plus pellet was estimated from days 6, 8 and 11 faeces samples as 8.7kg, 3.8kg and 4.5kg/head respectively when all sampled plant species were included separately in the analysis. The pellet (~1kg/day) comprised 4%-66% of the estimated daily intake of the individual heifers. The reason for this variability was unclear. These total intakes were low estimates and suggest that animals may have been grazing plant species that were not collected or there were problems with the marker technology or analyses as described above. The most likely marker-related problem is that the daily allowance of labelled supplement was all consumed between 1am and 9am each day, as animals ate the supplement as soon as they could. Such rapid consumption could perturb the gut flow of supplement markers, and hence affect the steady state of herbage markers.

The prototype bin is ready for others to use, however programming work is underway to: 1) develop a remote bin data download option, and 2) improve the intake estimates by providing the option of spreading supplement intake out more evenly during each 24 hour period by splitting the daily labelled supplement allowance into four 6 hour periods. This will be done by re-zeroing accumulated individual intakes more frequently and setting lower maximum allowed intakes for each of the 6 hour (re-zeroing) time intervals.

If these results are considered acceptable by interested parties, a proposal will be drafted to develop the bin system to the next stage by: 1) estimating the repeatability of pasture intake in animals across the year in 4 seasons, 2) measuring the effect of pasture intake on methane production across the year on 1 property, and 3) developing a two bin system to service 40 animals using a central process logic controller to monitor and control the daily supplement totals for the individuals.

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1. Background

Pasture feed intake by individual grazing cattle is very difficult to measure or estimate. Technically, the most successful approach to measure pasture intake has been to use indigestible, natural, plant markers, such as alkanes, recovered in faecal samples. However controlled release devices to deliver alkanes are no longer manufactured. An alternate approach of feeding weighed amounts of wax-labeled supplement to dose animals was developed (Charmley and Dove 2007). We proposed to develop a more practical approach by enabling the animal to self-dose with markers in the paddock via a purpose-built feed bin with EID tag reader, manufactured by Proway Livestock. The bin has mechanisms to control and record the daily beeswax-labeled supplement intake of each animal.

The similarity in patterns of alkanes extracted from cattle faeces and the herbage consumed was commented on by Oro et al. back in 1965 but the importance of these findings for studies of intake and selection by animals went unnoticed. Leaf waxes from terrestrial plants have a characteristic odd-over-even number predominance in the long chain (C27-C35) *n*-alkane series (Eglinton and Hamilton, 1967). The alkanes of plant cuticular wax have been used as markers to estimate feed intake by herbivores since the 1980s (Mayes et al., 1986). Intake can be estimated from the faecal concentrations of two alkanes, one derived from herbage and one administered to the animals by oral dosing (Mayes et al., 1986; Dove and Mayes, 2005). The method requires the assumption that the recovery in faeces of each of these alkanes is equal and validation studies have shown this to be the case (Dove and Mayes, 2005). Thus the method can be used without having to know the marker recoveries in faeces, but equally it requires alkane dosing and its accuracy is directly related to the accuracy of the dose, which is given at a constant daily amount over 5-8 days (Dove and Mayes, 2005;Charmley and Dove, 2007). In our studies, multiple alkane and alcohol markers were measured and average marker faecal recoveries (from the literature) were assumed, that differ between markers.

Different plant species have different patterns of marker concentrations (e.g. Brosh et al., 2003; Bugalho et al., 2004; Dove and Mayes, 2005) and this has been used to estimate diet composition in housed animals (e.g. Brosh et al., 2003; Elwert and Dove, 2005; Charmley and Dove, 2007) and grazing animals (e.g. Bugalho et al., 2004; Osoro et al., 2007; Piasentier et al., 2007) using least-squares procedures (Dillon et al. 2002; Dove and Moore, 1995; Mayes et al. 1994; Newman et al., 1995). It has been recommended that the concentration of natural alkanes in the forage needs to exceed 50 µg/g DM in order to obtain accurate estimates of forage intake (Casson et al. 1990). Grasses frequently have higher C33 concentrations relative to C29 concentrations than temperate pasture legumes (Dove 1992; Dove and Mayes 1991), while deciduous tree species have more shorter-chain alkanes relative to grasses (Dove and Mayes, 1996). Smith et al. (2001) found there was more difference between the alkane concentrations of flower-head, leaf and stem of a plant species than between whole plant samples, with alkanes being highest in flower-head, followed by leaf then stem. However, Dove et al. (1996) found that plant species contributed much more to the variance in the concentrations of individual alkanes (85%) than did harvest date and plant parts.

Plants use two principal biosynthetic pathways to fix carbon (Bassham et al., 1950; Slack and Hatch, 1967). The most common C3 (Calvin-Benson) pathway is characterized by an initial CO_2 carboxylation to form phosphoglyceric acid, a 3-carbon acid. The C4 (Hatch-Slack) pathway has evolved a CO_2-concentrating mechanism in which CO_2 initially combines with phosphoenol pyruvate to form a 4-carbon acid, oxaloacetate. This CO_2-concentrating mechanism gives C4 plants a competitive advantage under low CO_2 concentration conditions. Warm-season grasses and sedges use the C4 pathway, while virtually all trees, most shrubs, herbs, cool-season grasses and sedges use the C3 pathway. Long-chain n-alkanes produced by C3 and C4 plants show characteristically different $\partial^{13}C$ values (Collister et al., 1994). Bezabih et al. (2011) found different carbon isotopic enrichments of different alkanes in different plants and suggested that this could be used to improve diet composition estimations. Chen at al. (1998) had earlier shown that principal component and discriminant analyses could distinguish the patterns of alkanes in species occurring on both degraded, annual/short lived perennial pastures and improved perennial pastures.

The accuracy of estimation of diet composition, and thus forage intake, can be increased in diets containing phalaris by using other cuticular wax markers, such as the long-chain alcohols (LCOH) (Dove and Charmley, 2008). LCOHs also help distinguish between grasses and clovers. Vogts et al. (2007) reported that C3 savannah plants compared to C4 grasses had higher levels of C28 and C30 alcohols, while C4 grasses had higher levels of C32 alcohols and C33 alkanes.

In estimating diet composition in animals which are also consuming a feed supplement, the supplement can be treated as one of the diet components (Dove et al., 2002; Elwert and Dove, 2005; Charmley and Dove, 2007). If diet composition can be estimated and the actual intake of the supplement is known, then the intake of all other dietary components can be estimated (Dove et al., 2002; Elwert and Dove, 2005). This ‘labelled-supplement’ approach has the major advantage of not requiring separate alkane dosing. It requires knowledge of the faecal recoveries of the alkanes used to estimate diet composition and assumes that the faecal recovery of a given alkane is the same for each of the different dietary components (Elwert et al., 2008). Charmley and Dove (2007) found intake precision declined in sheep when faecal recoveries were based on treatment mean or grand mean recoveries rather than measured faecal recoveries from individual sheep, as did Morais et al. (2011) with cattle. The loss of precision by using grand means was improved by measuring both alkanes and LCOH (Dove and Charmley, 2008). Faecal recoveries of alkanes (Brosh et al., 2003; Bugalho et al., 2004; Ferreira et al., 2007, 2010; Morais et al., 2011; Olivan et al., 2007) and LCOH (Ali et al., 2004, 2005; Fraser et al., 2006) vary between diet types and intake level.

The accuracy and precision of the least squares intake solutions for each animal is affected by the sampling and measurement precision of the plant and faecal marker concentrations. Smith and Rickland (2007) reported that the intra-assay coefficients of variation for the gas chromatography/mass spectrometry (GC/MS) analysis of annual ryegrass, subterranean clover and bovine faeces alkanes ranged from 0.1%-12.9%, where lower concentrations of n-alkane produced a higher degree of imprecision caused by non-complete extraction. Measured faecal marker concentrations will also be affected by the accuracy of the faecal recoveries assumed for each marker if recovery is not measured in each animal, which is likely to be the case if the technique is applied in commercial situations.

Dove (2010) noted that when there were more markers available than there are possible species in the diet the least-squares procedures can accommodate information about all available alkanes, but this may not be a sensible approach because it is possible that some alkanes may discriminate much better than others between components. Dove et al. (1999) noted that other alkanes may prove to be negatively correlated with the capacity to distinguish between dietary components (Dove et al., 1999). Dove (2010) suggested in such cases, more reliable estimates of diet composition may be obtained if certain alkanes are not used in the calculation (Dove et al., 1999; Lewis et al., 2003; Lin et al., 2007). Theoretically, these would be the alkanes that contribute substantially to the discrepancy between observed and expected concentrations, but little to the actual discrimination of diet components. Dove (2010) suggested the issue was how to identify these and that this requires resolution if plant wax components are to gain wider acceptance as a method for estimating diet composition and intake. Dove (2010) noted that multivariate statistical procedures, such as discriminant analysis, could be used to establish whether plant species can be distinguished and which alkanes are best related to the ability to discriminate between diet components (Dove et al., 1999; Piasentier et al., 2000; Bugalho et al., 2004; Charmley and Dove 2007; Lin et al., 2008). Dove suggested that it would be a useful exercise to use such statistical procedures to explore whether it is mathematically likely that the diet components can be better discriminated. Cottle and Romero (in prep.) have modelled different weighting approaches and this project used some of the suggested improved least squares approaches from this modelling to estimate feed intake.

2. Project Objectives

The Project objectives were to:

1. Determine the accuracy of the system;
2. Determine the maximum number of head that could be serviced per bin and test cost
3. Determine the repeatability of supplement and chaff intake level, and
4. Submit a stage 2 proposal to MLA, if stage I results are successful.

Proway Livestock assisted with the project and they had the additional, related objective of developing a prototype feed bin system to successfully deliver controlled, measured amounts of (beeswax-labelled) supplement to individual cattle each day to enable the estimation of pasture feed intake from markers. The project objectives could obviously not be met without first meeting Proway's objective.

Many technical difficulties, e.g. power supply problems, necessary indicator programming changes, bin, door and race redesign, were met and solved during the project. The first prototype bin with a mechanical intake limiting device did not work satisfactorily and the bin had to be completely redesigned and rebuilt.

Objective 1 Accuracy of system

The accuracy of the system was estimated by individual cattle eating chaff through autofeeders in the Tullimba feedlot to obtain an estimate of 'actual' chaff intake and eating beeswax labelled supplement in the Proway-Cottle-Dove bin ('the bin') to obtain actual supplement intake. These actual intakes were then compared to their 'predicted' intake estimated by measuring markers in the feed (chaff and supplement) and faeces on three different days. Two chaffs were used, lucerne chaff and a mixed chaff (50% lucerne: 50% oaten). It should be noted that the actual intakes measured by the autofeeders in the feedlot were not reliable. Infrared beam interruption was not always triggered by animals entering and leaving the feed race and raw bin weight data had to undergo manipulation by algorithms developed by AGBU staff. The algorithms that were used to convert autofeeder bin weight and EID data into intake data used bin weight change inputs that were classed into three categories of diminishing levels of confidence that the weight changes were associated with a specific individual EID. The most likely actual intake result was considered to be the sum of these three categories (J Cook, pers. comm). Therefore there was some unavoidable doubt about the actual individual chaff intakes each day and these were highly variable between days. The autofeeders' lack of reliability is reflected by the recent installation of new Growsafe feeders at Tullimba. Thus it was not possible to estimate the accuracy of the system, rather the correlation of the two intake estimates are presented to demonstrate that intake predictions were in a feasible range based on the liveweights of the animals and the high (rank) correlation of intake measured by autofeeder versus markers.

Pasture intake was also predicted while animals were grazing a native pasture. This predicted pasture intake could only be compared with the intake calculated from their liveweight and expected rate of liveweight gain, as there is no benchmark standard for measuring pasture intake.

2.1. Prototype bin 1: lucerne chaff

Methods

On 19 May 2011, 28 Angus Hereford cross cattle arrived at Tullimba feedlot and were placed on lucerne chaff fed from a feed bunker. On 30 May, 27 steers eating chaff were moved into two pens, each with an autofeeder, with an adjoining back lane for joint access to a supplement bin feeder that was located in one of the two pens. One autofeeder contained lucerne chaff and one contained mixed chaff and the chaff was available *ad lib*. Detailed records were then kept of all chaff intakes for as long as cattle were in the autofeeder pens. The first prototype supplement bin feeder arrived on 6 June. It contained a mechanical device to limit the amount of supplement that could be consumed based on equipment used in existing commercial Proway supplement bins. After determining that daily recorded intakes of the two chaffs were very inconsistent between and within steers and after a series of bin technical difficulties, a lucerne chaff only run was conducted from 17 June – 30 June with 20 steers. Faecal samples were taken from the 20 steers on 30 June.

Chaff and faecal samples were dried at 65 degrees in an oven for at least 7 days before being ground through a sieve (Figure 1). The dried, ground samples were sent to the CSIRO, Canberra laboratory where markers were analysed following the methods of Dove and Mayes (1996).

Figure 1. Dried ground faecal samples.

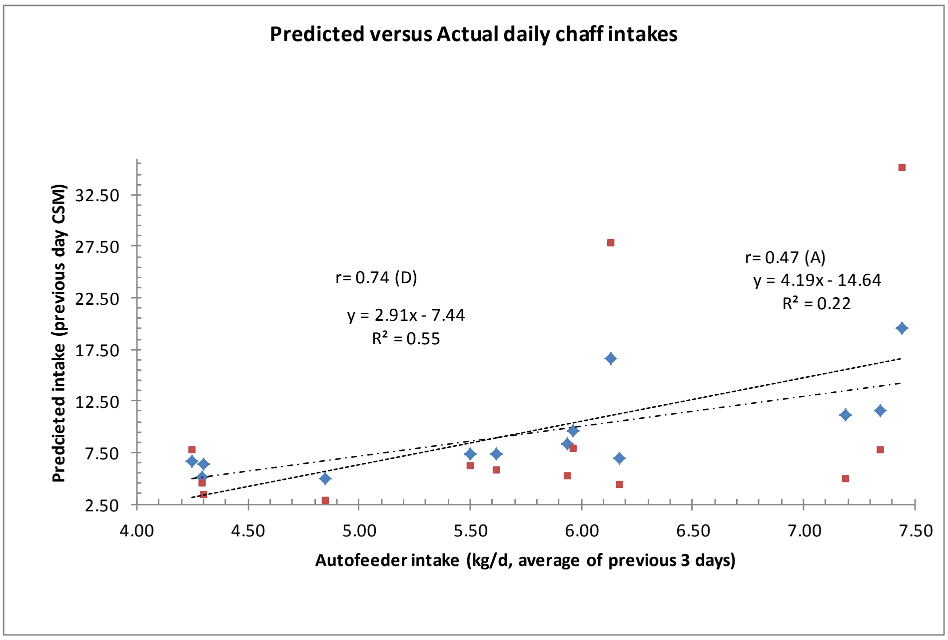
The marker results were then analysed by Xcel/solver using coding written by Prof. Cottle. In the least squares analysis (Dove and Moore 1995) markers were either not weighted, i.e. all weighted by 1 (Method A) or weighted using weights based on a canonical discriminant analysis of z score weights (Method D - Cottle and Romero, submitted).

Results and Discussion

Average chaff intake was 5.8 kg/day. Repeatability of daily chaff intake was 0.27. The prototype bin was unsuccessful in restricting supplement intake to 1kg/head/day. For example, steer WIL18 ate between 2.68 kg and 6.96kg CSM/day with the access gap set at its minimum. A decision was taken to manufacture a new bin with a more sophisticated and robust mechanism to restrict supplement intake to 1kg/head/day.

The correlation (r) between predicted OMI and autofeeder chaff intake was 0.74 for animals with more consistent supplement intakes using method D (Figure 2). Method D (discriminant marker weightings) predicted intakes were more highly correlated with autofeeder chaff intakes than non-weighting of markers. Predicted intakes were higher than autofeeder intakes.

Figure 2. Predicted versus actual daily chaff intakes of steers. Method A estimates (red) follows the standard practice of not differentially weighting markers. Method D (blue) uses weights from a canonical discriminant analysis of a large plant marker database (Cottle and Romero, submitted).



2.2. Prototype bin 2: pasture

Methods

On 22 September a new lot of 24 Angus-Charolais cross heifers were purchased. The heifers were fed chaff while waiting for the second prototype Proway bin to arrive. On 19 November the heifers were moved to an adjacent native pasture paddock as there was still no sign of the second bin arriving. On 23 January the second prototype bin arrived (Figure 3).

Figure 3. Second prototype bin with automatic gate with a shorter race.

Amongst the bin modifications was the addition of a ram-driven, blocking gate at the bottom of the bin that allows the supplement intake of individual animals to be better controlled. The Rinstrum indicator FEED menu items that can now be user-controlled are:

- T.LIM (Time limit): The maximum time that an animal can occupy the feeder before the gate closes. Measured in minutes.
- WEIGHT (Weight Limit): The maximum weight that an animal can eat per day, before it is blocked.
- RST.HR (Reset Hour): The hour of the day that the daily animal feed weight totals are cleared, e.g. 1h means 1am.
- IDLE (Idle action): When there is no animal at the feeder, is the gate open or closed.
- G.CLOSE (Gate close time): The time required for the gate to close. Measured in 0.1 seconds.
- T.BLOCK (Cow block time): If an animal has been blocked, how long after the animal departs will the feeder unblock. Measured in seconds.

The parameters were set at: Time limit reduced from 3 to 1 minute, weight 1kg, RstHr at 1am, idle at allow/ gate open, G.close set at 5 seconds, T.Block set at 15 seconds.

The heifers were moved back to the feedlot bunker on 28 January to more easily train the heifers to take supplement from the bin and then placed in the pens and fed chaff. A series of technical difficulties (relay switching, defective solar charging unit etc.) were then experienced with the new bin that were reported in detail in project progress reports. On 10 February the cattle and bin were moved back to the paddock. The bin was then re-engineered, including changing how weights are recorded, re-programming and adding an infrared eye to the race. A series of new bin technical difficulties were then experienced and resolved.

March 9 was considered day 0 of the trial as the bin had been working as desired administering steady amounts of supplement for a few days. On March 14 pasture samples were taken and the **day 5 faecal sample** was taken, despite various data problems, including a few rows of bin weight data being missed due to intermittent power supply problems, so the subsequent pen trials could be completed in time for other demands upon the feedlot facility. On March 16 the **day 7 faecal sample** was taken and March 19 the **day 10 faecal sample** was taken.

Samples and results were analysed as described previously.

Results and Discussion

The main pasture species sampled in the paddock (with the %DM of as-is wet weight) were:

Wallaby grass (37% DM)

Red grass (41% DM)

White clover (29% DM)

Setaria (36% DM)

Parramatta grass (43% DM)

Paspalum (32% DM)

The cottonseed meal (CSM) pellets (4mm) contained 0.75% (w/w) (yellow) beeswax and 30% oat hulls. Extracted CSM itself contains only trace amounts of the markers. Of the 10 (common) markers chosen for inclusion in the analysis, the beeswax contained unusually high levels of C25 and C27 alkane, C24 and C28 alcohol and unusually low levels of C31 and C33 alcohols (Tables 1 and 2, Figures 3-5). This unique marker profile, particularly of alkanes, helps to better distinguish the beeswax labelled CSM supplement from the chaff or pasture components of the diet. It was recognised that the CSM intakes of some heifers were incorrect for a short period where 20 errant rows of data occurred in the bin, where exit bin weights were higher than entry bin weights for an inexplicable reason. It was not possible to know which animals data were affected.

A principal components analysis was then conducted as an *a priori* tool to address the question "are we likely to be able to discriminate between pasture species, or will there likely be problems with data analysis?". The PCA analyses of alkanes and alcohols are shown in Figures 4-6.

Figure 4. Clustering of plant species based on the first two principal components of alkane marker concentrations.

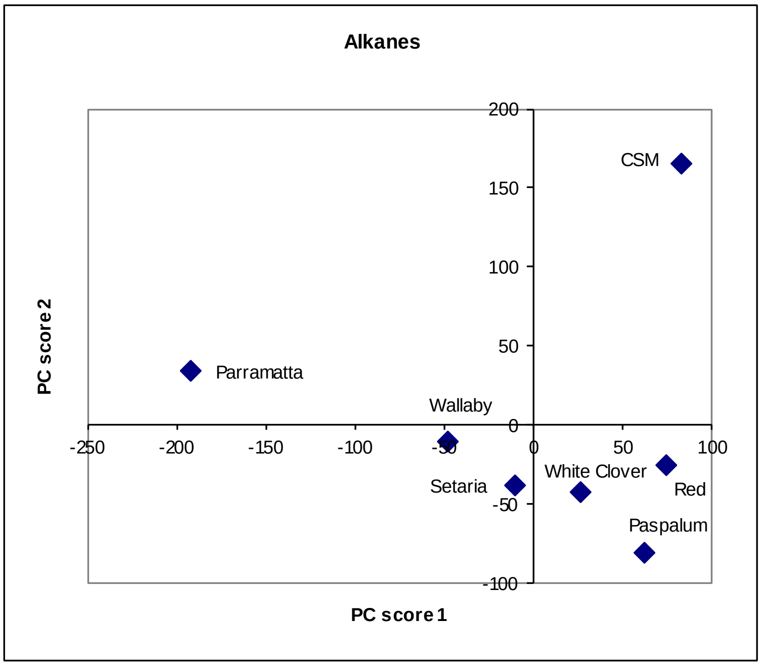


Figure 5. Clustering of plant species based on the first two principal components of alcohol marker concentrations.

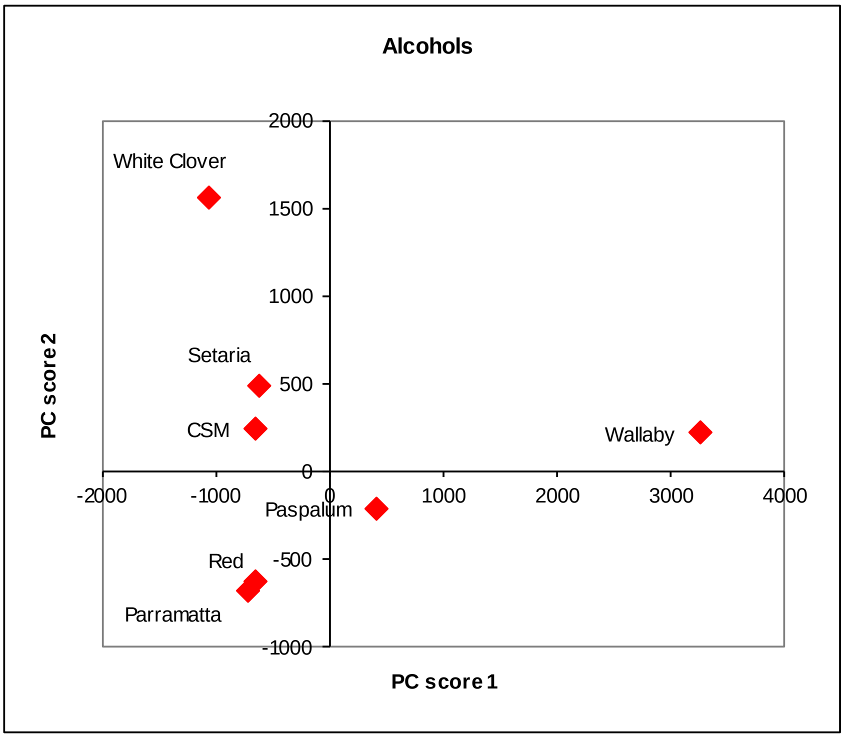
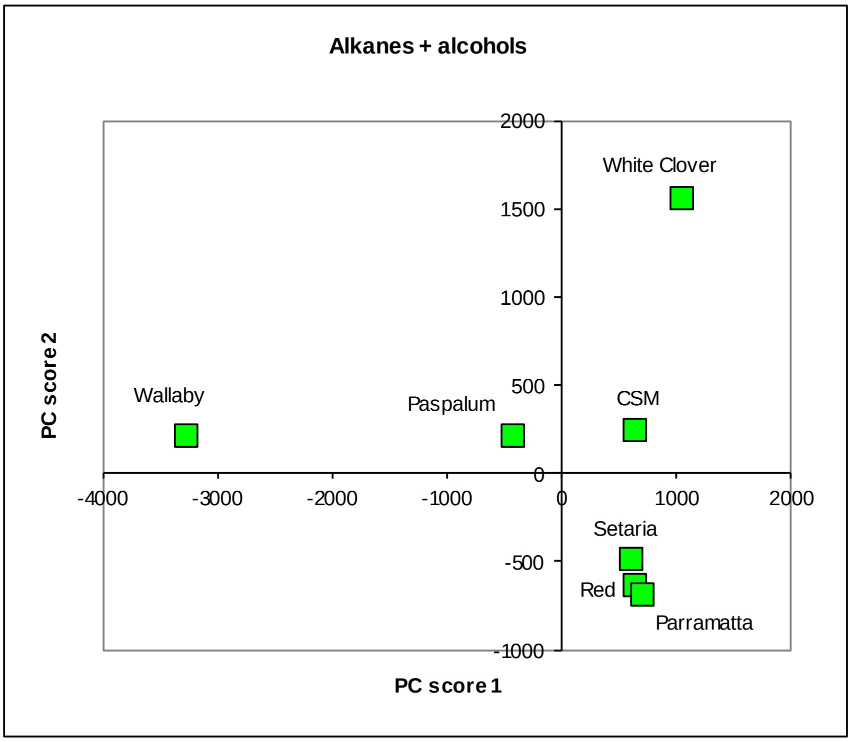


Figure 6. Clustering of plant species based on the first two principal components of alkane and alcohol marker concentrations.



Results of the PC analyses (Figure 6) suggested that even with alkanes and alcohols included together, CSM would be distinct but we were likely to have problems separating red grass, setaria and paramatta grass. We therefore decided to bulk all grasses into a single ‘grass’ fraction as well as analyse the plants separately, expecting the former approach to be more successful. The marker concentrations of feed samples from the all three runs (pasture, lucerne and mixed chaff) are shown in Tables 1 and 2.

The heifers grazed on a native pasture with very diverse plant species with some improved species conveniently located adjacent to the feedlot. In a commercial situation it would be better advised to graze test animals on an improved pasture with fewer plant species. It is not possible to know the true intake of pasture. However if total intake is estimated by using the equation of Minson and McDonald (1987), then assuming a liveweight gain of 0.25kg/day in winter, the daily intake of the heifers should have been about 11kg/day, as they had an average liveweight of 455kg in late April 2012. A subsample of 5 heifers that were weighed had gained at 0.25kg/day. As the heifers were allocated about 1kg CSM pellet per day, their intake of pasture was therefore expected to be about 10kg/day.

The estimated intakes when the average marker concentration of individual grasses was used, as suggested by the PCA analyses are shown in Tables 3, 4 and 5. The estimated pasture intakes when all grasses were combined and an average marker concentration was used are shown in Tables 6, 7 and 8. Many different methods of analyses were run, including using raw marker concentrations, but only results from transformed marker concentrations are presented and discussed in this report. Faecal samples are usually homogenous and only small samples are usually taken in many research trials.

Table 1. Alkane concentrations in feed (ppm/OM) and DM and OM content.

The markers in bold, larger font are those used in the 8 markers analyses.

	Alkanes												
	C23	C25	C26	**C27**	C28	**C29**	C30	**C31**	C32	**C33**	DM%	ash	OM%
	Pasture run												
CSM pasture	16.67	52.69	7.44	226.18	7.09	154.47	5.70	125.71	7.94	22.44	89.25	0.0655	83.40
	Lucerne run												
CSM Pellet Day 1		57.98	7.58	233.69	7.27	151.87	5.73	120.57	6.37	21.13	89.12	0.0644	83.38
CSM Pellet Day 5		66.71	8.34	265.76	7.74	169.71	6.18	132.56	4.97	22.00	90.26	0.0653	84.37
CSM Pellet day 8		63.67	7.90	252.50	7.57	160.72	5.89	127.02	5.75	21.11	89.31	0.0643	83.57
CSM Pellet Day 11		58.50	7.52	242.60	7.33	159.58	5.97	126.84	5.75	21.37	90.11	0.0629	84.44
	Mixed chaff run												
CSM pellet day 4	32.71	100.72	12.44	418.44	11.79	253.79	8.38	203.43	6.38	31.07	90.00	0.0772	83.05
CSM pellet day 6	31.42	96.76	12.31	401.67	11.55	244.39	8.61	196.10	5.74	30.77	89.11	0.0770	82.25
CSM pellet day 9	31.79	101.51	12.49	430.56	11.41	266.26	8.65	211.90	6.07	33.45	89.79	0.0778	82.80
CSM pellet day 11	33.82	107.17	13.52	447.98	12.16	274.42	9.08	216.08	6.30	34.82	90.48	0.0781	83.41
Lucerne Chaff Day 1		11.10	3.39	41.67	10.34	256.84	17.51	392.65	13.02	23.17	89.09	0.0706	82.80
Lucerne Chaff Day 5		12.38	3.15	45.33	7.55	238.72	15.41	366.70	11.83	22.52	88.01	0.0900	80.09
Lucerne chaff day 8		5.53	2.95	16.78	5.77	100.37	10.00	386.92	16.94	35.68	86.07	0.0799	79.19
Lucerne Chaff Day 11		11.59	3.58	40.36	9.65	222.27	15.54	347.15	11.75	20.72	90.64	0.0848	82.95
Mixed chaff day 4		21.55		43.25	10.09	172.69	12.32	284.75	11.81	53.09	89.77	0.0716	83.34
Mixed chaff day 6		19.27		36.60	8.56	151.89	11.72	296.24	11.77	53.85	89.19	0.0724	82.73
Mixed chaff day 9		19.75		41.14	9.90	164.04	12.04	269.03	12.23	52.80	88.16	0.0754	81.51
Mixed chaff day 11		12.11		30.31	7.15	147.85	12.57	376.16	14.89	45.39	90.40	0.0797	83.20
Wallaby Grass	4.52	11.53		24.99	3.93	103.48	6.14	166.36	6.91	82.97	94.59	0.0789	87.13
Red Grass	6.09	13.87	3.92	77.15	5.65	66.61		52.93	5.23	39.08	95.15	0.0548	89.94
White clover		5.49		19.46	4.98	101.99	7.30	98.33	7.72	37.77	94.42	0.0761	87.23
Setaria	3.92	9.19	3.61	52.85	4.77	64.67	3.97	79.14	5.63	134.37	94.73	0.0628	88.78
Parramatta Grass		11.96	3.77	42.13	5.87	73.08	9.59	276.86	17.75	191.69	94.60	0.0556	89.34
Paspalum	3.65	8.06		22.57	3.73	35.81	3.21	58.48	4.56	23.45	94.97	0.0612	89.16

Table 2. Alcohol concentrations in feed (ppm/OM).

The markers in bold, larger font are those used in the 8 markers analyses.

	Alcohol									
	C18OH	**C19OH**	**C21OH**	**C22OH**	**C23OH**	**C24OH**	**C25OH**	**C26OH**	**C28OH**	**C30OH**
	Pasture run									
CSM pasture	39.68	39.13	20.69	46.05	12.73	326.62	4.26	261.27	357.59	1047.36
	Lucerne run									
CSM Pellet Day 1	12.37		13.66	44.60		307.46		249.53	337.17	856.73
CSM Pellet Day 5	11.84		14.84	52.53		329.99		264.69	352.51	892.51
CSM Pellet Day 8	11.51		12.92	46.78		312.92		268.19	341.79	841.03
CSM Pellet Day 10	11.58		14.81	50.54		318.06		254.28	338.20	818.02
	Mixed chaff run									
CSM pellet day 4	19.48	23.93		101.02				455.96	477.00	995.50
CSM pellet day 6	25.31	30.41		111.82	9.47	611.13	2.55	443.66	456.94	916.19
CSM pellet day 9	22.64	36.51		77.59	8.27	543.55		421.66	549.14	1315.21
CSM pellet day 11	31.32	33.06		101.29	10.67	638.31	2.85	483.02	511.17	1059.77
Lucerne Chaff Day 1	30.43		16.74	48.57		50.94		116.76	175.84	2312.12
Lucerne Chaff Day 5	14.95		13.66	50.85		55.14		148.47	177.31	2152.56
Lucerne chaff day 8	22.55		15.96	28.16		36.44		39.68	90.75	1918.94
Lucerne Chaff Day 11	24.57		14.76	61.09		61.77		154.94	164.13	2085.65
Mixed chaff day 4	26.32	39.21		31.71	11.27	44.06	11.67	1364.32	216.31	931.20
Mixed chaff day 6	25.91	31.55		29.76	10.15	40.54	11.85	1384.07	156.37	894.26
Mixed chaff day 9	25.99	27.41		32.42	8.85	39.00	12.18	1385.89	148.83	864.38
Mixed chaff day 11	28.50	35.10		40.65	9.00	44.53	9.25	941.95	122.76	1268.52
Wallaby Grass	45.83	46.80	22.13	14.60	13.89	142.77	4.99	90.60	4174.36	155.61
Red Grass	42.74	31.58	7.63	20.29	11.96	53.87	5.53	40.07	169.25	211.14
White clover	45.30	31.50	20.23	61.75	10.34	64.81	6.36	180.97	258.81	2436.54
Setaria	34.50	31.85	18.00	21.99	10.85	43.25	7.83	87.47	231.61	350.10
Parramatta Grass	31.63	32.62	17.94	31.77	12.15	88.29	13.67	213.27	91.61	170.52
Paspalum	39.20	32.45	22.71	10.88	11.47	35.73	4.79	50.43	1307.71	380.82

Any marker technique can be expected to be less successful when the animals' intakes are variable during the marker supplementation and plant and faecal sampling periods. Faecal sampling is recommended on day 6-8 after markers have been eaten at a consistent rate.
When grasses were combined the average daily intake of pasture plus pellet was estimated from days 5, 7 and 10 faeces samples as 7.8kg, 6.9kg and 9.7kg/head respectively (Tables 6-8). The percentage of the estimated daily intake of the individual heifers that was made up of the CSM pellet varied widely. The source of this variability is unknown. The total intakes appear to be low estimates (i.e. lower than 11kg/day) and suggest that animals may have been grazing plant species that were not collected. This was quite possible and more likely than there being problems with the marker technology, given the hundreds of research papers published on using markers to estimate intake.

When the pasture samples were first collected for a pasture run that did not eventuate because of technical problems with the bin (see methods), there was a large patch of self-sown demeter fescue (with easy to identify seed heads) in the paddock that the cattle appeared to preferentially graze. When the pasture was sampled for the actual pasture run a few weeks later the fescue was not sampled as it was harder for the author to identify the plant as it was no longer in seed. In hindsight, this may have been a mistake as interestingly, when literature values for demeter fescue marker concentrations were included in the analysis, the pasture intakes estimated were closer to those expected with the labelled CSM being 8.3±1.77% of the diet with an implied total intake of 12.0 kg and whole-diet digestibility of 75.6%. The average estimated diet composition included 14% fescue in the diet, which may have been the case.

The most likely marker related problem is that, according to the bin data, the daily allowance of labelled supplement was all consumed, by all animals, between 1am and 9am each day, as animals appeared to eat the supplement as soon as they could. Such behaviour will cause a disjunct between the gut flow of markers derived from the pasture, cf. those derived from the CSM.

As a result of the Principal Components Analyses the data for alkane and alcohol concentrations for the grass species were averaged across grass species to derive a marker profile for a pooled 'grass component' (Tables 6-8). Strictly speaking, such averaging should be weighted for the proportions of each grass species in the sward, but such data were not available.

When the marker data were not transformed it is possible to calculate the implied mean OM digestibility of the consumed diet, as well as calculating intake. These values (70-76%) were consistent with the nature of the pasture on offer.

When data are not transformed or weighted that same results were obtained when data were run processed by EatWhat software. Overall the most credible results were obtained when data were transformed and weighted. This accords with the theoretical modelling results of Cottle and Romero (submitted).

Table 3. Estimated intake of dietary components (OMI) from the day 5 faecal sample.

a) 8 markers, transformed, weighted (average intake 8.7 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total	*CSM%
6502	1.48	0.00	0.00	0.00	0.00	0.00	0.98	2.46	40%
6503	2.30	0.00	0.00	0.00	0.00	0.66	0.99	3.95	25%
6504	2.56	0.00	0.00	0.00	0.00	1.58	0.83	4.97	17%
6505	2.85	0.00	0.00	0.00	0.00	0.20	0.70	3.75	19%
6506	5.07	0.00	0.00	0.00	0.41	7.59	1.05	14.13	7%
6513	2.60	0.27	0.34	0.00	1.34	2.20	0.82	7.56	11%
6515	2.95	0.00	0.00	0.00	0.00	0.00	1.20	4.15	29%
6518	8.54	0.00	0.00	0.00	0.00	16.50	0.95	26.00	4%
6519	0.70	0.00	0.00	0.00	0.00	0.00	1.03	1.73	59%

b) 8 markers, transformed, not weighted (average intake 10.9 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6502	1.17	0.00	0.00	0.00	0.18	0.21	0.98	2.54
6503	1.66	0.00	0.00	0.00	0.50	2.19	0.99	5.34
6504	2.42	0.00	0.00	0.00	0.70	1.48	0.83	5.43
6505	2.76	0.00	0.00	0.00	0.41	0.05	0.70	3.90
6506	5.23	0.00	0.00	0.00	2.79	9.56	1.05	18.64
6513	1.98	0.00	0.41	0.00	1.42	1.61	0.82	6.24
6515	2.84	0.00	0.00	0.00	0.00	0.00	1.20	4.04
6518	9.56	0.00	0.00	0.00	5.29	23.23	0.95	39.04
6519	0.36	0.00	0.00	0.00	0.00	0.44	1.03	1.83

c) 10 markers, transformed, weighted (average intake 7.4 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6502	1.10	0.00	0.00	0.00	0.17	0.30	0.98	2.55
6503	1.65	0.00	0.00	0.00	0.39	1.57	0.99	4.61
6504	2.33	0.00	0.00	0.00	0.70	1.66	0.83	5.52
6505	2.57	0.00	0.00	0.00	0.34	0.00	0.70	3.60
6506	4.31	0.00	0.00	0.00	2.05	7.12	1.05	14.53
6513	1.74	0.00	0.34	0.00	1.39	1.87	0.82	6.15
6515	2.70	0.00	0.00	0.00	0.00	0.00	1.20	3.90
6518	5.49	0.00	0.00	0.00	2.45	10.42	0.95	19.31
6519	0.34	0.00	0.00	0.00	0.00	0.44	1.03	1.81
6521	1.02	0.00	0.00	0.00	0.00	0.00	1.28	2.30

d) 10 markers, transformed, not weighted (average intake 9.0 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6502	1.17	0.00	0.00	0.00	0.16	0.15	0.98	2.46
6503	1.70	0.00	0.00	0.00	0.48	1.84	0.99	5.01
6504	2.35	0.00	0.00	0.00	0.66	1.42	0.83	5.27
6505	2.65	0.00	0.00	0.00	0.37	0.00	0.70	3.72
6506	4.91	0.00	0.00	0.00	2.56	8.38	1.05	16.90
6513	1.92	0.00	0.38	0.00	1.35	1.46	0.82	5.93
6515	2.83	0.00	0.00	0.00	0.00	0.00	1.20	4.02
6518	8.09	0.00	0.00	0.00	4.32	17.45	0.95	30.82
6519	0.39	0.00	0.00	0.00	0.00	0.38	1.03	1.80
6521	1.04	0.00	0.00	0.00	0.00	0.00	1.28	2.32

*Percentage of diet made up of pellet intake. WG: wallaby grass, RG: red grass; WC: white clover; PG: Parramatta grass

Table 4. Estimated intakes of dietary components (OMI) from the day 7 faecal sample.

a) 8 markers, transformed, weighted (3.8 kg/day)

Animal	Wallaby Grass	Red Grass	White clover	Setaria	Parramatta Grass	Paspalum	CSML1	Total	*CSM%
6502	1.16	0.00	0.00	0.00	0.02	0.00	0.96	2.13	45%
6503	1.16	0.00	0.00	0.00	0.28	0.32	0.97	2.73	36%
6504	3.56	0.00	0.00	0.00	0.51	0.89	0.90	5.86	15%
6505	2.72	0.00	0.00	0.00	0.32	0.73	0.84	4.61	18%
6515	2.25	0.00	0.00	0.00	0.00	0.00	1.13	3.38	33%
6519	0.39	0.00	0.00	0.00	0.00	0.00	0.76	1.15	66%

*Percentage of diet made up of pellet intake

b) 8 markers, transformed, unweighted (7.5 kg/day)

Animal	Wallaby Grass	Red Grass	White clover	Setaria	Parramatta Grass	Paspalum	CSML1	Total
6502	1.19	0.00	0.00	0.00	0.01	0.00	0.96	2.16
6503	1.13	0.00	0.00	0.00	0.32	0.52	0.97	2.94
6504	3.67	0.00	0.00	0.00	0.55	0.89	0.90	6.01
6505	2.99	0.00	0.00	0.00	0.45	1.25	0.84	5.53
6513	8.90	2.56	0.00	0.00	3.46	8.95	0.58	24.45
6515	2.41	0.00	0.00	0.00	0.00	0.00	1.13	3.53
6519	0.40	0.00	0.00	0.00	0.00	0.00	0.76	1.16

c) 10 markers, transformed, weighted (average intake 6.9 kg/day)

Animal	Wallaby Grass	Red Grass	White clover	Setaria	Parramatta Grass	Paspalum	CSML1	Total
6502	1.15	0.00	0.00	0.00	0.02	0.00	0.96	2.12
6503	1.15	0.00	0.00	0.00	0.27	0.31	0.97	2.70
6504	3.50	0.00	0.00	0.00	0.50	0.83	0.90	5.73
6505	2.68	0.00	0.00	0.00	0.31	0.69	0.84	4.52
6506	0.64	0.00	0.00	0.00	0.03	0.00	0.83	1.50
6513	10.15	3.29	0.00	0.00	3.82	10.53	0.58	28.37
6515	2.32	0.00	0.00	0.00	0.00	0.00	1.13	3.44
6519	0.39	0.00	0.00	0.00	0.00	0.00	0.76	1.15
6521	2.64	0.00	0.00	0.00	0.27	0.90	1.00	4.80

d) 10 markers, transformed, not weighted (average 4.6 kg/day)

Animal	Wallaby Grass	Red Grass	White clover	Setaria	Parramatta Grass	Paspalum	CSML1	Total
6502	1.17	0.00	0.00	0.00	0.01	0.00	0.96	2.14
6503	1.16	0.00	0.00	0.00	0.30	0.40	0.97	2.83
6504	3.56	0.00	0.00	0.00	0.53	0.73	0.90	5.71
6505	2.91	0.00	0.00	0.00	0.43	1.04	0.84	5.21
6506	0.66	0.00	0.00	0.00	0.04	0.00	0.83	1.53
6513	3.51	0.51	0.00	0.09	1.26	3.13	0.58	9.08
6515	2.44	0.00	0.00	0.00	0.00	0.00	1.13	3.57
6519	0.40	0.00	0.00	0.00	0.00	0.00	0.76	1.16
6521	2.68	0.00	0.00	0.00	0.26	0.78	1.00	4.72

Table 5. Estimated intakes of dietary components (OMI) from the day 10 faecal sample.

a) 8 markers, transformed, weighted (average intake 4.5 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total	*CSM%
6501	0.65	0.00	0.00	0.00	0.18	0.61	1.41	2.86	49%
6502	2.02	0.00	0.00	0.00	0.10	0.14	0.60	2.86	21%
6503	3.64	0.00	0.00	0.00	0.97	0.93	1.09	6.63	16%
6504	1.05	0.00	0.00	0.00	0.06	0.00	0.42	1.54	27%
6505	2.71	0.00	0.00	0.00	0.47	0.00	0.58	3.75	15%
6506	2.57	0.00	0.00	0.00	0.00	0.00	0.96	3.54	27%
6513	3.21	0.99	0.32	0.00	2.02	2.92	0.60	10.06	6%
6515	1.63	0.00	0.00	0.00	0.00	0.00	1.20	2.83	42%
6517	2.02	0.00	0.00	0.00	0.11	0.00	0.53	2.67	20%
6519	1.63	0.00	0.00	0.00	0.10	0.00	0.88	2.61	34%

b) 8 markers, transformed, unweighted (average intake 4.6 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6501	0.70	0.00	0.00	0.00	0.19	0.58	1.41	2.88
6502	2.07	0.00	0.00	0.00	0.10	0.13	0.60	2.91
6503	3.94	0.00	0.00	0.00	1.14	1.45	1.09	7.62
6504	1.07	0.00	0.00	0.00	0.08	0.33	0.42	1.89
6505	3.17	0.00	0.00	0.00	0.64	0.73	0.58	5.12
6506	2.76	0.00	0.00	0.00	0.00	0.00	0.96	3.72
6513	2.21	0.35	0.26	0.00	1.25	1.67	0.60	6.34
6515	1.68	0.00	0.00	0.00	0.00	0.00	1.20	2.88
6517	2.49	0.00	0.00	0.00	0.17	0.76	0.53	3.94
6519	1.66	0.00	0.00	0.00	0.08	0.00	0.88	2.62

c) 10 markers, transformed, weighted (average intake 4.2 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6501	0.66	0.00	0.00	0.00	0.17	0.57	1.41	2.81
6502	1.96	0.00	0.00	0.00	0.08	0.03	0.60	2.67
6503	3.65	0.00	0.00	0.00	1.00	1.07	1.09	6.81
6504	1.04	0.00	0.00	0.00	0.06	0.00	0.42	1.52
6505	2.69	0.00	0.00	0.00	0.47	0.03	0.58	3.77
6506	2.60	0.00	0.00	0.00	0.00	0.00	0.96	3.57
6513	2.85	0.89	0.24	0.00	1.79	2.59	0.60	8.96
6515	1.63	0.00	0.00	0.00	0.00	0.00	1.20	2.82
6517	2.08	0.01	0.00	0.00	0.12	0.00	0.53	2.75
6519	1.62	0.00	0.00	0.00	0.10	0.02	0.88	2.61
6521	1.02	0.00	0.00	0.00	0.00	0.43	0.51	1.96

d) 10 markers, transformed, not weighted (average 4.0 kg/day)

Animal	WG	RG	WC	Setaria	PG	Paspalum	CSML1	Total
6501	0.74	0.00	0.00	0.00	0.18	0.48	1.41	2.80
6502	2.04	0.00	0.00	0.00	0.10	0.12	0.60	2.86
6503	3.87	0.00	0.00	0.00	1.12	1.39	1.09	7.46
6504	1.05	0.00	0.00	0.00	0.05	0.00	0.42	1.52
6505	3.32	0.00	0.00	0.00	0.72	1.06	0.58	5.68
6506	2.76	0.00	0.00	0.00	0.00	0.00	0.96	3.72
6513	1.59	0.00	0.18	0.02	0.88	1.18	0.60	4.45
6515	1.67	0.00	0.00	0.00	0.00	0.00	1.20	2.87
6517	2.11	0.00	0.00	0.00	0.11	0.00	0.53	2.75
6519	1.64	0.00	0.00	0.00	0.07	0.00	0.88	2.59
6521	1.18	0.07	0.00	0.00	0.00	0.14	0.51	1.90

*Percentage of diet made up of pellet intake. WG: wallaby grass, RG: red grass; WC: white clover; PG: Parramatta grass

Table 6. Estimated intakes of dietary components (OMI) from the day 5 faecal sample. Grasses combined. WC: white clover

a) 8 markers, transformed, weighted (average intake 8.0 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.09	0.00	0.98	3.07
6503	3.98	0.00	0.99	4.98
6504	5.57	0.00	0.83	6.40
6505	11.76	0.00	0.70	12.45
6506	8.64	0.00	1.05	9.69
6513	4.22	0.65	0.82	5.69
6515	7.40	0.00	1.20	8.59
6518	9.85	0.00	0.95	10.80
6519	0.86	0.00	1.03	1.89
6521	0.85	0.00	1.28	2.13

b) 8 markers, transformed, not weighted (average intake 7.8 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.02	0.00	0.98	3.00
6503	3.80	0.00	0.99	4.79
6504	5.06	0.01	0.83	5.90
6505	9.42	0.00	0.70	10.12
6506	9.27	0.20	1.05	10.52
6513	4.73	0.86	0.82	6.41
6515	7.41	0.00	1.20	8.61
6518	9.64	0.11	0.95	10.70
6519	0.87	0.00	1.03	1.90
6521	0.87	0.03	1.28	2.18

c) 10 markers, transformed, weighted (average intake 7.8 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.05	0.00	0.98	3.03
6503	3.93	0.00	0.99	4.93
6504	5.39	0.00	0.83	6.22
6505	11.28	0.00	0.70	11.97
6506	8.27	0.00	1.05	9.32
6513	3.85	0.56	0.82	5.23
6515	7.79	0.00	1.20	8.99
6518	9.53	0.00	0.95	10.48
6519	0.86	0.00	1.03	1.89
6521	0.84	0.00	1.28	2.12

d) 10 markers, transformed, not weighted (average intake 7.7 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.01	0.00	0.98	2.99
6503	3.80	0.00	0.99	4.79
6504	4.97	0.00	0.83	5.80
6505	9.54	0.00	0.70	10.23
6506	8.66	0.13	1.05	9.84
6513	4.44	0.77	0.82	6.03
6515	7.80	0.00	1.20	9.00
6518	9.30	0.07	0.95	10.32
6519	0.87	0.00	1.03	1.90
6521	0.85	0.01	1.28	2.14

Table 7. Estimated intakes of dietary components (OMI) from the day 7 faecal sample. All grasses combined. WC: white clover.

a) 10 markers, transformed, weighted (6.9 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.05	0.00	0.96	3.01
6503	2.08	0.00	0.97	3.05
6504	14.43	0.00	0.90	15.33
6505	8.97	0.00	0.84	9.81
6506	0.97	0.00	0.83	1.80
6513	4.87	0.03	0.58	5.49
6515	5.74	0.00	1.13	6.87
6519	0.55	0.00	0.76	1.31
6521	7.82	0.00	1.00	8.81

b) 10 markers, transformed, not weighted (6.6 kg/day)

Animal	Grass	WC	CSML1	Total
6502	2.04	0.00	0.96	3.00
6503	2.07	0.00	0.97	3.05
6504	12.01	0.00	0.90	12.91
6505	8.23	0.00	0.84	9.07
6506	1.00	0.00	0.83	1.83
6513	5.56	0.18	0.58	6.32
6515	5.97	0.00	1.13	7.10
6519	0.56	0.00	0.76	1.33
6521	7.13	0.00	1.00	8.12

Table 8. Estimated intakes of dietary components (OMI) from the day 10 faecal sample. All grasses combined. WC: white clover.

a) 10 markers, transformed, weighted (9.7 kg/day)

Animal	Grass	WC	CSML1	Total
6501	1.45	0.00	1.41	2.85
6502	10.44	0.00	0.60	11.04
6503	8.82	0.00	1.09	9.91
6504	3.00	0.00	0.42	3.42
6505	18.18	0.00	0.58	18.75
6506	10.33	0.00	0.96	11.29
6513	3.38	0.32	0.60	4.29
6515	3.12	0.00	1.20	4.32
6517	20.58	0.00	0.53	21.11
6519	3.57	0.00	0.88	4.45
6521	2.91	0.00	0.51	3.42

b) 10 markers, transformed, not weighted (8.7 kg/day)

Animal	Grass	WC	CSML1	Total
6501	1.47	0.00	1.41	2.87
6502	8.82	0.00	0.60	9.42
6503	8.15	0.00	1.09	9.24
6504	2.80	0.00	0.42	3.22
6505	12.28	0.00	0.58	12.86
6506	10.50	0.00	0.96	11.47
6513	3.78	0.45	0.60	4.82
6515	3.11	0.00	1.20	4.31
6517	18.00	0.00	0.53	18.53
6519	3.46	0.00	0.88	4.34
6521	2.85	0.00	0.51	3.36

2.3. Prototype bin 2: lucerne chaff

Methods

A series of new technical difficulties were experienced and solved with the bin. Use of 3 movement sensitive cameras determined that a longer race with a guard protected IR eye was needed to stop heifers parasitising other heifers' supplement. On April 5 the 14 heifers eating supplement consistently were moved to the chaff feed bunker. On April 7 the heifers were placed in pens and fed lucerne chaff. On April 16 the Proway bin was working without problems for the first time and this was recorded as day 1 of the lucerne chaff run using a new cottonseed meal (CSM) supplement containing 0.75% beeswax and 30% oat hulls. On April 20 the **day 5 faecal** sample was taken, on April 23 the **day 8 faecal** sample was taken, on April 26 the **day 11 faecal** sample was taken and mixed chaff was fed in afternoon.

Samples and results were analysed as described previously. The actual daily chaff intakes were averaged for the 2-4 days before the faecal sampling day, while the plant marker concentrations were averaged from the analysis of chaff and supplement samples taken from the bins immediately before and on the faecal sample day.

Various weightings of markers were carried out on the transformed marker concentrations to obtain least squares intake solutions, including: 1) no weighting (Method A), the standard method; 2) weighting by discriminant weights from a z score analysis (Method D), and 3) weighting by a (confidential) parameter.

Method 3 was overall the most successful, in terms of producing the highest correlations between actual and predicted chaff intakes, and these are the results presented in the following graphs. Further study may further improve the weighting method used. All predicted intakes were in OM units so were divided by the average OM% of the relevant feed samples to be compared to actual as-is wet weight intakes. That is, all feed intake results are shown as 'as-is' weight eaten.

Results and Discussion

The repeatability of autofeeder daily lucerne chaff intake, determined from an analysis of variance of between and within animal intake, was 0.14. The correlation between actual chaff intake (the average of autofeeder chaff intakes on the 2-4 days before faecal sampling) and chaff intake predicted from the marker concentrations in the faeces are shown in Figures 6-8. Figure 7 has heifer 6515's data removed. Figures 8 and 9 have heifer 6504's data removed as they were outliers with predicted intakes more than 4kg higher than their autofeeder intake. The correlation varied from 0.63-0.96. For the range of autofeeder intakes of 9-14kg/day, the predicted intake values were 8-19kg.

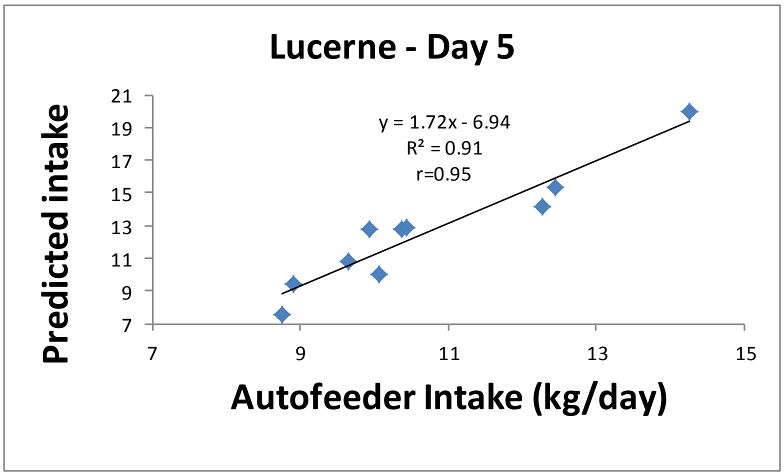
When using 8 markers, the correlations when all transformed marker concentrations were weighted by 1 were 0.94, 0.44 and 0.77 respectively for faecal sample taken on days 5, 8 and 11, so the standard method worked well on day 5 and day 11 data, but not as well on day 8 data.

Daily feed intake on pasture can be is expected to be much more consistent than chaff intake in a feedlot pen with a single race autofeeder, where some shyer feeders only ate every second day due to the presence of more aggressive feeders in the pen.

The marker predicted lucerne chaff intakes based on all faecal samples were higher than the autofeeder intakes for the range of intakes in the trial (Figures 7-9). As the actual intakes were unknown, it is not possible to say which intake estimate was more accurate.

Figure 7. Predicted versus actual lucerne chaff intake (kg/head/day) from day 5 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) Using 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)

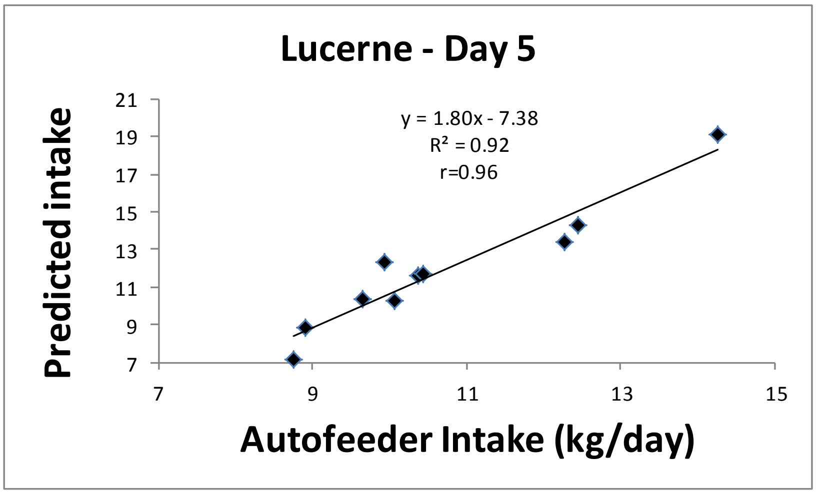
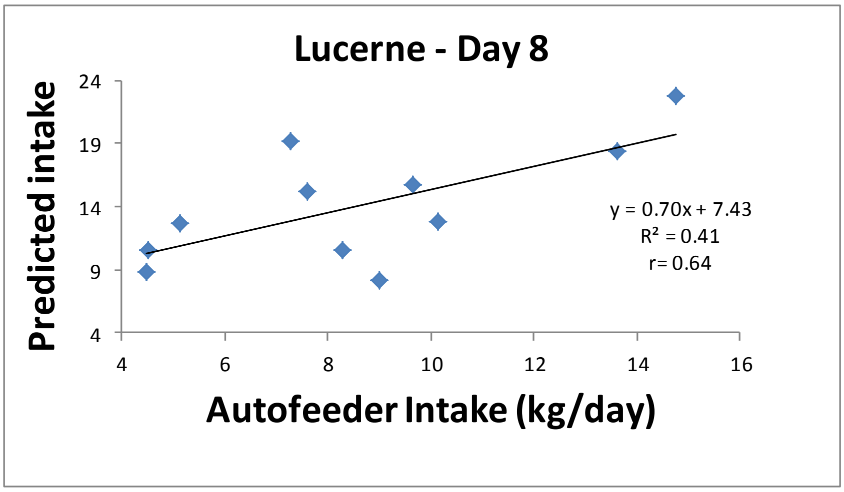


Figure 8. Predicted versus actual lucerne chaff intake (kg/head/day) from day 8 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) Using 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)

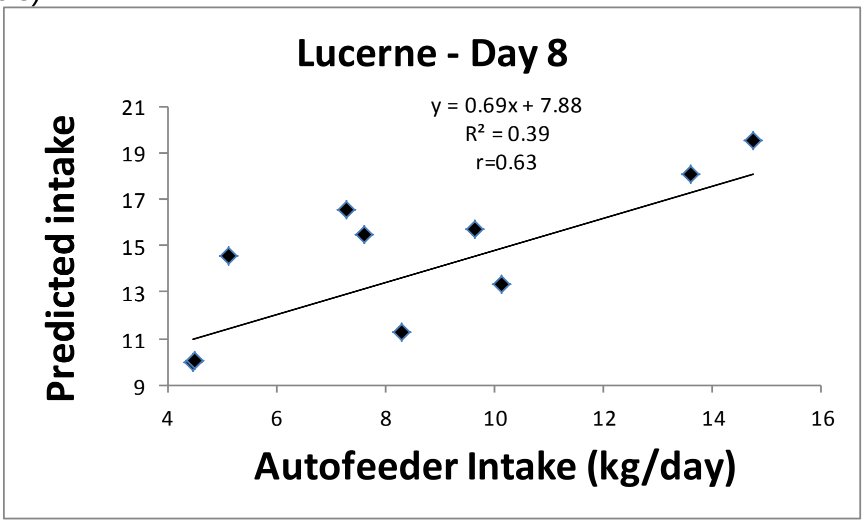
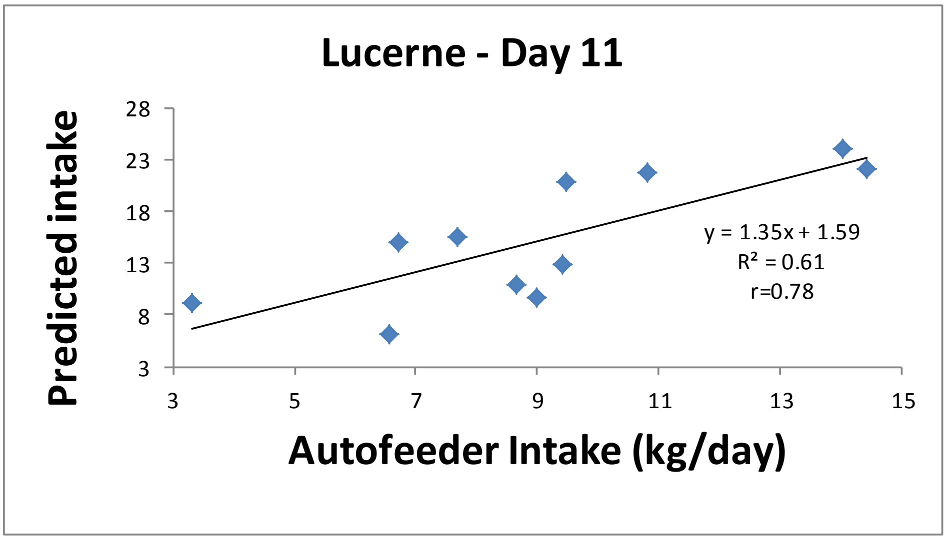
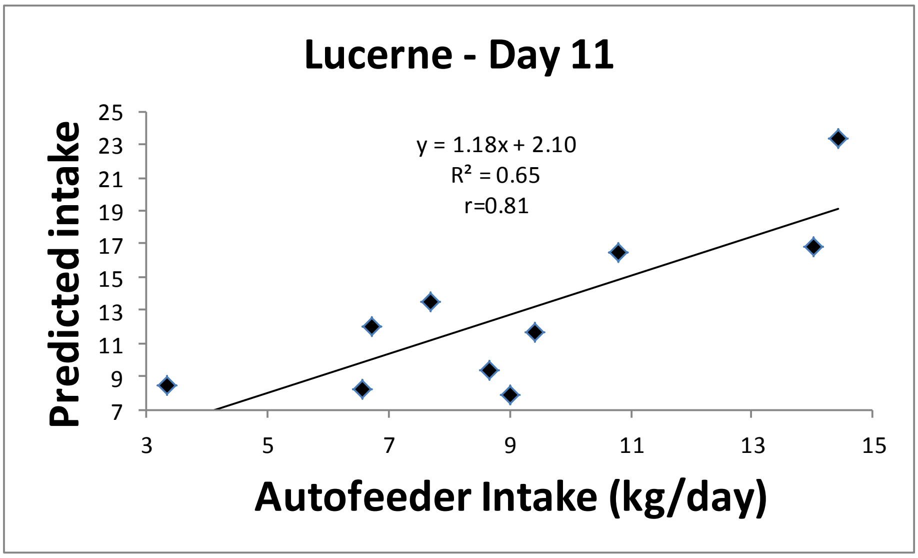


Figure 9. Predicted versus actual lucerne chaff intake (kg/head/day) from day 11 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) Using 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



2.4. Prototype bin 2: mixed chaff

Methods

On April 29 the original CSM supplement (1.25% wax) was fed and this was recorded as **day 1** of the mixed chaff run**.** On May 4 the **day 6 faecal** sample was taken, on May 7 the **day 9 faecal** sample was taken and on May 9 the **day 11 faecal** sample was taken.

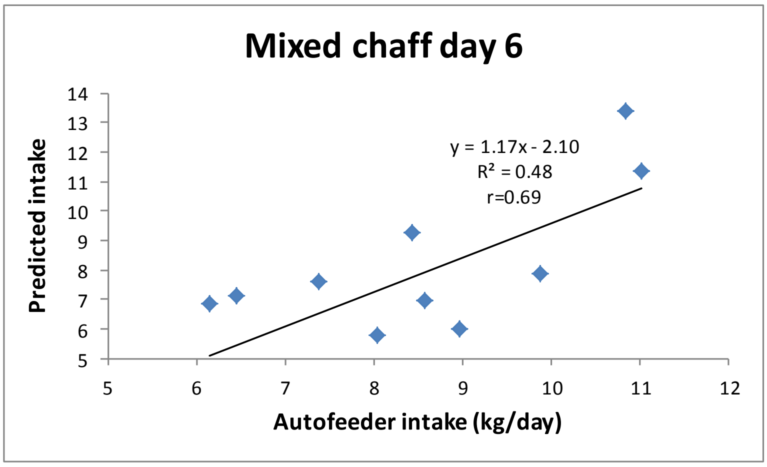
Samples and results were analysed as described previously. The cottonseed meal (CSM) pellets (4mm) contained 1.25% (w/w) (yellow) beeswax.

Results and Discussion

The repeatability of autofeeder daily mixed chaff intake was 0.10. The correlation between actual mixed chaff intake (the average of autofeeder chaff intakes on the 2-4 days before faecal sampling) and chaff intake predicted from the marker concentrations in the faeces are shown in Figures 10-12. Figure 10 had heifer 6502's data removed.

Figure 10. Predicted versus actual mixed chaff intake (kg/head/day) from day 6 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) Using 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)

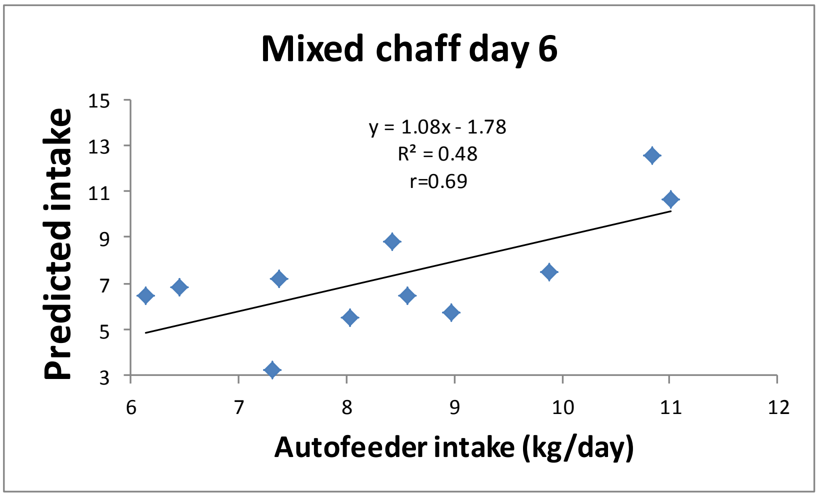
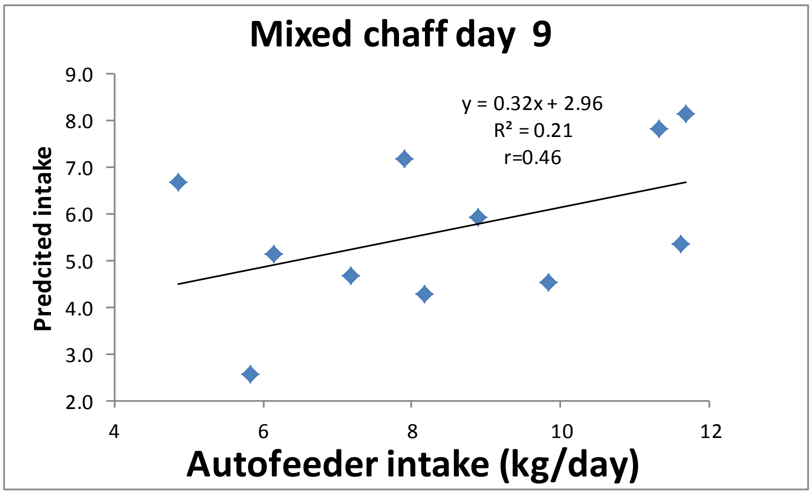


Figure 11. Predicted versus actual mixed chaff intake (kg/head/day) from day 9 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) Using 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)

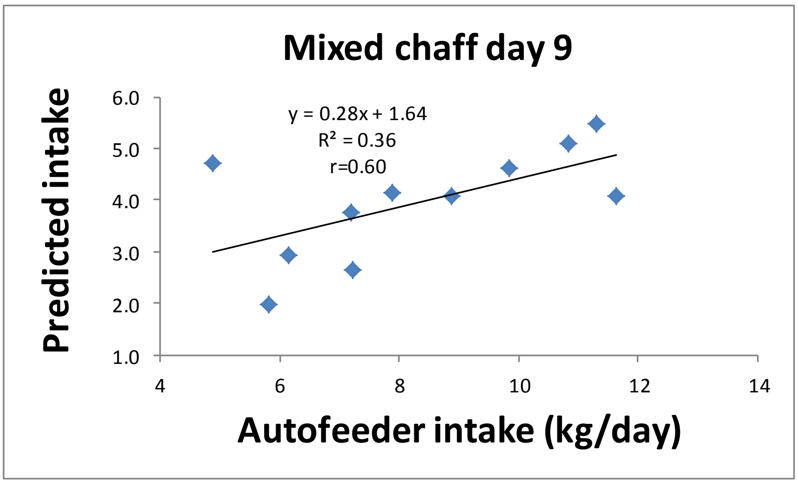
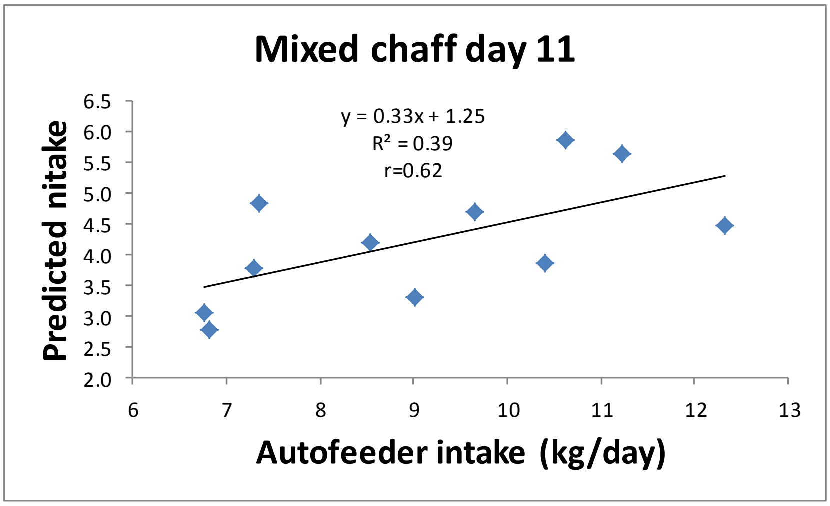
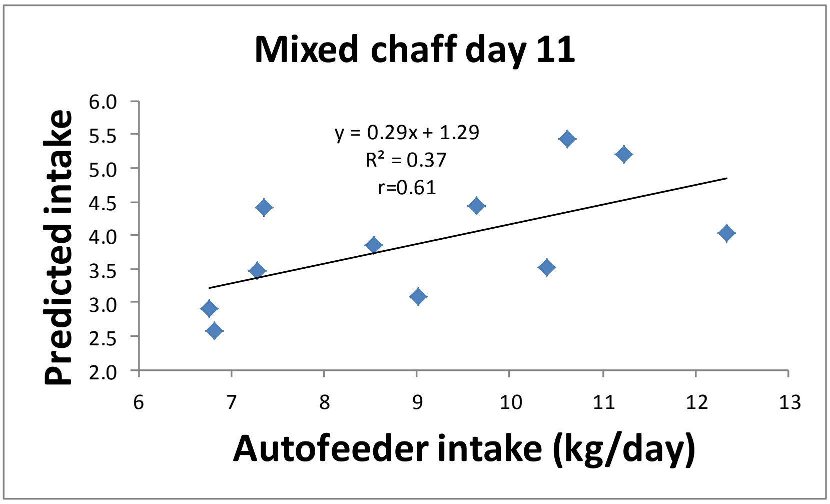


Figure 12. Predicted versus actual mixed chaff intake (kg/head/day) from day 11 faecal sampling.

a) Using 8 markers (C27, C29, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



b) *Using* 10 markers (C25, C27, C29, C30, C31, C33 alkanes; C24, C26, C28, C30 alcohols)



The correlation varied from 0.46- 0.69 which was lower than occurred with the lucerne chaff run. The 'actual' daily mixed chaff intakes were less repeatable and more variable (49% CV for 11 days) than lucerne chaff intakes (37% CV for 11 days), which could help explain the lower correlation. That is, it may not have been due to marker technology problems.

In contrast to the lucerne chaff results, the marker predicted mixed chaff intakes based on all faecal samples were lower than the autofeeder intakes for the range of intakes in the trial (Figures 10-12). As the actual intakes were unknown, it is again not possible to say which intake estimate was more accurate.

Using 8 markers, the correlations when all transformed marker concentrations were not weighted were 0.71, 0.36 and 0.59 respectively for faecal samples taken on days 6, 9 and 11, which was slightly lower than when differentiating weights were used. However, 3 cattle had to have their predicted intake results removed before analysis for day 6 and 9 faecal sampling analyses, as very low proportions of their diet were estimated as consisting of cottonseed meal, resulting in unrealistically high predicted chaff intakes. There were therefore problems with analysing data generated from two of the three sampling days. The more palatable a supplement and the more training of cattle to supplement, the less likely supplement intake would be a problem with future use of the system.

Objective 2 Maximum number of head per bin and test cost

The prototype bin 1 results (Figure 2) demonstrated that a Proway bin could service 20 head effectively eating *ad lib* supplement. The new prototype bin should be able to service at least 20 head. It is difficult to know the upper limit as it depends very much on the social dynamics of different mobs of cattle. Numbers of heifers eating from the second prototype bin was probably reduced due to the lengthy periods of the bin being empty of supplement while technical bin problems were being addressed.

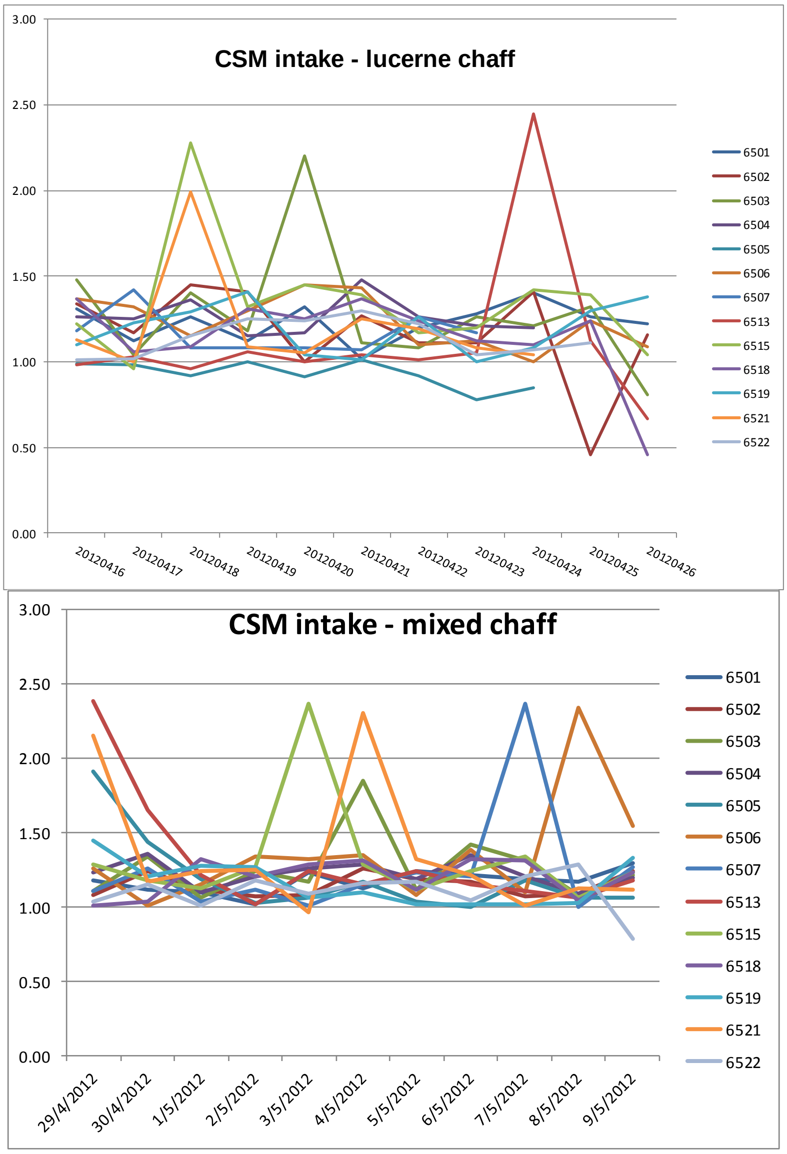
In a commercial system, cattle that have not been fed supplement from a bin before may have to be trained in yards as part of the system. Cattle go off feed when the weather is undergoing change. It is not known if the cattle would have taken to the supplement as quickly if they had remained in the paddock versus in the pen, where cattle commenced eating supplement within 2 days. Training in yards would unfortunately add to the cost of obtaining a pasture feed intake measurement, as additional supplement will have to be fed during any training period and the bin will need to be moved from yards to the relevant paddock for subsequent pasture intake measurement. The need for training on to supplement was not part of the original experimental design and is a possible future student research project. Presumably it will depend on the feeding history of the cattle. Angus-Charolais cross heifers at pasture on another property were given access to 20kg supplement in a trough in a yard and immediately took to the supplement.

It will be recommended to confine cattle on test to a small area (e.g. by hot tape and tread-ins) adjacent to a main watering point with the bin(s) about 200m from the water. Removal of all cattle that initially eat supplement after a few days with their reintroduction a few days later will encourage shy feeders (who usually then continue to eat).

Objective 3 Repeatability of supplement and chaff intake

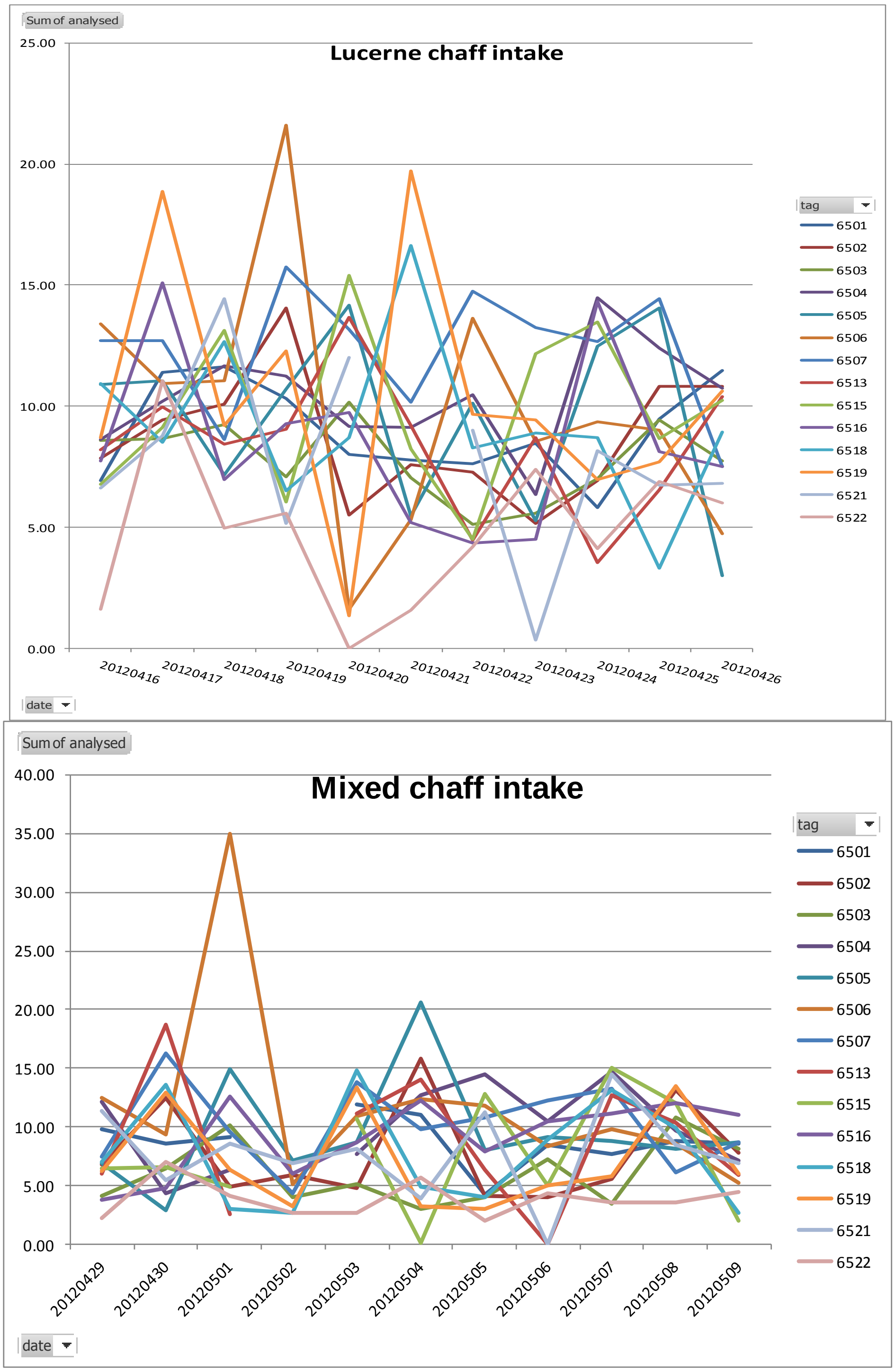
Repeatability of supplement intake from the first prototype bin was low (0.12). For example, WIL18 ate between 2.68 kg and 6.96kg CSM/day, a much larger range than steers eating less CSM. The consistency of CSM intake is now much higher with the second prototype bin (daily supplement repeatabilities over 0.9) as maximum CSM intake is better restricted and varies less between days (Figure 13). Greedy animals occasionally eat more in their allotted 54 seconds if they are still below the set daily maximum intake when they enter for their final allowed session. Some animals appear to learn to do this.

Figure 13. Intake of CSM during the lucerne and mixed chaff runs.



Repeatability of chaff intake in feedlot pens was consistently less than 0.1 (see methods section and Figure 14). This makes difficult the choice of which days' intakes to use or estimate in a trial run. In run 1.2 the 3 days chaff intake and 1 day's CSM intake before faecal sampling were used. Cattle in feedlot pens with auto-feeders may gorge one day and not eat the next. This probably does not occur so much at pasture where repeatability of intake is probably much higher, although given the lack of published estimates of the repeatability of pasture feed intake of cattle this can only be conjecture. However, chaff intake in the Tullimba feedlot did not appear to simulate grazing behaviour very well for most animals.

Figure 14. Actual lucerne and mixed chaff intake (kg/head/day).



Objective 4 Submission of a funding proposal for stage 2

An ARC linkage application, based on stage 2, was submitted for funding to commence in July 1 2012. ARC rules did not allow MLA to be a leverage partner. The proposal was not funded. Proway bins were also included in activity 6 of Dr Robert Herd's round 1 DAFF application that MLA is co-funding. The proposal has been funded but activity 6 was removed and recommended for submission to DCCEE's July methodology funding round. However the MDP funding does not allow for development of technologies.

Prof. Cottle and Dr. Herd have applied for DAFF Filling the Research Gaps round 2 funding to use a Proway Livestock/Sapien Technology four bin system to measure pasture intake in high and low methane producing cows at Trangie. This project has also not been funded. Sapien are now working with Proway to overcome the following limitations of the Rinstrum technology:

1. The equipment is only capable of controlling a few bins. Larger mobs will require several bins operating together.
2. Power consumption is relatively high and requires additional expenditure on relatively high capacity remote power supplies (batteries, solar panels etc).
3. The equipment is not capable of remote operation or management.
4. Unit costs per bin are high and there is no opportunity to reduce per bin costs. The single bin technology solution is in the vicinity of $15,000 to $16,000.

A multi-bin solution requires many bins in close proximity to be a commercially viable solution for taking to the market. Sapien Technology is overcoming the limitations described above with a combination of strategies.

1. Using existing technology and intellectual property that they have designed and developed such as their cloud database platforms and RFID power saving technology. The cloud database platform will be developed for exporting data to Breedplan.
2. Using existing technology that they license/procure such as their remote access technologies and RFID synchronisation and multiplexing.
3. Using their in depth technical knowledge of RFID, scales and microprocessor control for a bin controller platform that optimises costs, power and eliminates the issues with the system used in this project.

3. Recommendations

The project provided valuable input into the design of the Proway bin with it now functioning as desired in a single bin configuration. Most single bin technical issues have been resolved with some issues, e.g. multi-bin systems, ultrasonic movement detection, about to be developed.

The Proway bin system should be further developed by:

- estimating the repeatability of pasture intake in animals across the year in 4 seasons
- measuring the effect of pasture intake on methane production across the year
- developing a multi bin system to service more animals using a central PLC to monitor and control the daily supplement intakes by individuals before developing larger multi-bin systems.

A proposal related to these recommendations has been submitted to DAFF by Prof. Cottle and adjunct Professors Herd and Oddy, for round 2 Filling the Research gaps funding. In any future pasture R&D measuring pasture intake, the paddocks should be selected to contain fewer pasture species, e.g. improved pastures, as the marker technology requires a larger number of markers than number of grazed pasture species to obtain an intake solution. The bin system would not be appropriate for use with diverse, native pastures where 5-6 plant species do not account for nearly all of the animals' pasture intake.

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6. Commercial Interest

Proway Livestock has commercial agreements in place with UNE and Murrulla Meat.