



**Final Report to HCC**

**On**

**Effective Utilisation of Protein in Silages made  
from grass/clover leys for Beef and Sheep  
Production**

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## **Project Outline**

**The strategic action plan for the Welsh red meat industry has the vision of supporting the development of a profitable, efficient, sustainable and innovative Welsh red meat industry. The use of home-grown forages on livestock farms in Wales is central to achieving this vision, as it can improve farm profitability whilst reducing the environmental foot print of production and improving product quality. However, currently the accurate analysis of many silages produced on Welsh farms could be standing in the way of accomplishing this goal. The following project provides a way forward to address this short coming.**

**Background** The success of the work done at IBERS on research and development of red and white clover varieties has led to widespread adoption of grass/clover swards throughout Wales and the ensiling of these in both organic and conventional systems. Clovers fix atmospheric nitrogen, reduce N fertilizer requirements and give a high protein forage. Not only does this have positive environmental benefits but also delivers huge cost savings in reducing fertilizer and purchasing protein supplements from outside Wales.

For the range of silages produced it is essential to have accurate analysis if the supplementation of conserved winter forages is to be done efficiently.

Silage analysis is based on the near infra red spectroscopy (NIR) which relies on analyses of silages of known quality as the reference point. The main research work on this technique for grass silages was done in the 1990's and although modifications have been introduced no in depth studies of clover rich silages have taken place. Consultants working across Wales have become increasingly concerned that the NIR analysis is not giving reliable estimates of the crude protein concentrations in silages containing significant proportions of white or red clover. These silages would be expected to contain in the range of 14-18% protein, but many NIR analyses are indicating levels of 10-14%. This is leading to over supplementation with costly protein concentrates. The reasons for this perceived poor analysis is likely to be due to the data base of standard silages used in the original calibration work, not being sufficiently comprehensive or representative to include high clover silages which have become incorporated into many farm silage making practices today.

**Aims and Objectives** This project had the key aim of quantifying the extent of the problem of the accuracy of analysis of silages that contain clover (bale or clamp). The report focuses on assessing the validity and the extent of the perceived inaccuracy of the analyses and the potential influence this could have on the inefficient use of home grown forages. The report identifies what questions warrant further investigation. The report's findings will also be used to educate the industry on how best to analyse clover rich silages in the immediate and long term. The findings will be used to provide information for inclusion in a variety of farmer orientated communications.

## **Project Procedures**

The project had the key remit of understanding and quantifying the difference between NIR analysis, the standard on-farm analysis conducted in the UK today, and the more accurate and traditional wet chemical approaches. The use of silages from beef and sheep farms that have variable levels of clover inclusion in their swards was decided upon as a key test to the NIR analysis, as these silage types are likely to fall at the extremes of the types of samples that are used to calibrate the NIR technique.

In order to fulfil the desired project brief in a timely but also challenging way for the analytical services the following procedures were decided upon and for the reasons highlighted.

1. Common problems with silage analyses are
  - a. Time delay between sample collection and analysis
  - b. Variation in analyst and other variables (scientific equipment, reagent shelf life etc) with time, ie month to month variability.

For these reasons it was decided to sample all farms in a 36 h period and transport all samples to the laboratory the following day. This should result in low analytical variability across all samples and as such make the NIR/wet chemical analyses more 'similar'.

2. It was felt that Geographical distribution would be of less importance in providing wide sample variation. Thus farm selection was focused more on the important factors such as wide range of harvesting times, widest variation in bales compared to clamp as the ensiling methodology. Finally method of sample collection ie, cored versus grab, both procedures are used to provide samples for analysis in the 'real-world' and it was felt that it was important to provide variation in this process for this particular sample set.

With 1 and 2 above in mind the decision was made to limit the geographical areas to 3.

The 3 chosen areas were:-

North Ceredigion  
Montgomeryshire  
Breconshire

All farms were sampled on either the late afternoon (after 15.30) of Sunday January 2<sup>nd</sup> (2 farms), or Monday 3<sup>rd</sup> January according to the following procedure;

Arrive on farm.

Collect basic information about silage eg, cutting date, 1<sup>st</sup>, 2<sup>nd</sup> cut, additive use.

Collect sample (at least 1 kg) either by core or a grab from the face or open bale.

Place the whole sample in a large plastic container and mix well.

Sub-sample at least 500 g into two separate sample bags, 1 sample each for NIRs and wet chemistry.

Squeeze bags to remove as much air as possible prior to sealing.

Move on to the next sample/farm.

Table 1 gives a resume of the farms sampled and the sample information with respect to harvest date, silage type, sample collection method and estimated clover content.

Once collected the samples were taken to the laboratory. The samples were stored at 4°C until delivery to the laboratory. On January 4<sup>th</sup> the samples were transported by car to the commercial analytical laboratory that conducted both the Wet Chemical and NIR Analyses. They were passed over to the laboratory at approximately 14.00h

The laboratory was selected on 2 criteria

1. First and foremost it is part of the Forage Analysis Assurance (FAA) group and as such has to meet a high standard of analysis. The company also, to ensure industry-wide quality, has to participate in a number of ring tests run by the FAA. So ultimately all FAA laboratories have methodologies in place to calibrate their analyses.
2. It has a high throughput of samples and so is one, among a handful, of the main laboratories used for silage analysis in the UK.

The analyses conducted were

1. A standard NIR analysis giving the full range of silage chemical components
2. Wet chemical analyses for pH, Dry Matter, Crude Protein, Neutral Detergent Fibre, Lactic acid and Ammonia-N. The wet chemical analyses were conducted by recognized industry standard procedures.

**Table 1 – Resume of the silage samples collected**

<b>Sample No.</b>	<b>Approximate Harvest date</b>	<b>Silage Cut</b>	<b>Bale or clamp</b>	<b>Grab or Cored</b>	<b>Estimated Clover %</b>
1	21 <sup>st</sup> June	1st	clamp	Grab	5
2	Sept	2nd	bale	Cored	10
3	30th June	1st	clamp	Grab	10
4	6th June	1st	bale	Cored	30
5	end Aug	1st	bale	Grab	20
6	June 3 <sup>rd</sup> week	1st	clamp	Grab	5
7	June 3 <sup>rd</sup> week	1st	clamp	Grab	10
8	July	2nd	bale	Cored	30
9	May	1st	bale	Cored	20
10	June new field	1st	bale	Cored	15
11	18th July	1st	bale	Grab	5
12	mid Sept	1st	bale	Grab	0
13	1st week Aug	1st	bale	Grab	5
14	2nd week July	1st	bale	Grab	5
15	Aug	2nd	bale	Grab	10
16	16th June	1st	clamp	Grab	10
17	16th June	1st	clamp	Grab	10
18	July/Aug	1st	bale	Grab	10
19	July/Aug	1st	bale	Cored	10
21	Aug 10 <sup>th</sup>	2nd	bale	Cored	40
22	June 28 <sup>th</sup>	1st	bale	Cored	25
23	N/A	1st	bale	Cored	N/A
24	N/A	1st	bale	Grab	N/A
25	N/A	1st	bale	Grab	N/A
26	N/A	2nd	clamp	Grab	N/A
27	June	1st	clamp	Grab	10
28	June	1st	bale	Grab	10
29	Aug	2nd	bale	Grab	5
30	16th June	1st	clamp	Grab	5
31	July	2nd	bale	Grab	10
32	Mid June	1st	bale	Cored	10
33	Mid July	1st	clamp	Grab	10
34	Mid Aug	1st	bale	Grab	5
35	end June	1st	clamp	Grab	5
36	end June	1st	clamp	Grab	5
37	Mid June	1st	clamp	Grab	5
38	end Aug	2nd	bale	Cored	5
39	20th July	1st	bale	Grab	10
40	End Aug/ Start Sept	2nd	bale	Grab	5
41	20th June	1st	bale	Grab	5
42	20th June	1st	clamp	Grab	5
43	21st July	1st	clamp	Grab	5
44	end July	1st	bale	Grab	60
45	end July	1st	clamp	Grab	5
46	Mid June	2nd	clamp	Grab	10
47	Mid June	1st	clamp	Grab	10
48	Mixed 1 <sup>st</sup> /2 <sup>nd</sup> mid/June – End Aug	2nd	clamp	Grab	10

<b>Table 1 continued – Resume of the silage samples collected</b>					
<b>Sample No.</b>	<b>Approximate Harvest date</b>	<b>Silage Cut</b>	<b>Bale or clamp</b>	<b>Grab or Cored</b>	<b>Estimated Clover %</b>
49	end June	1st	clamp	Grab	10
50	Mid- June	1st	clamp	Grab	50
51	Mid-June	1st	clamp	Grab	50
52	Mid- June	1st	clamp	Grab	50
53	August	1st	clamp	Grab	50
54	Mid June	1st	clamp	Grab	5
55	July	1st	bale	Grab	5
57	Mid June	1st	clamp	Grab	10
58	21 <sup>st</sup> Aug	2nd	clamp	Grab	5
59	1st week June	1st	bale	Grab	5
60	mid Sept	2nd	bale	Grab	5

N/A = data not available

### **Data analysis**

Data were analysed using linear mixed models (the REML procedure of Genstat 13<sup>th</sup> Edition). Analysis type (wet chemistry or NIR) was the fixed effect, and sample number was the random effect. Initially, all data were included in the data analysis; subsequent analyses separated first and second cut silages (of both silage types), and baled versus clamped silages (of both cuts). The wet chemistry results were regarded as the ‘absolute’ values, and the effects of NIR analysis on the data were tested for significance. Statistical significance was declared at  $P < 0.05$ .

It should be noted that this comparison is only for this one wet chemistry laboratory and this one NIR analysis lab. This data analysis does not indicate how NIR analysis in general (as may be carried out by other labs) compares with standard analytical techniques (as carried out in other labs). For that comparison, replicate subsamples of silages would need to be analysed by a number of different labs using standard chemical analysis techniques and NIR analysis/prediction equations. However, as the main laboratories are all part of a ring test for their NIR analysis and are governed by the same analytical procedures/protocols, it is highly likely that the findings in this report give a good interpretation of the state of silage analyses in commercial laboratories across the UK.

### **Results and Discussion**

For the project to be of value it was first important to obtain a wide variation in sample type and composition that are found on Welsh sheep and beef farms. So it is worthy of noting from Table 1 that there was a wide range of cutting times from May through to September, however as would also be expected on those farm types in Wales the majority of samples were harvested between mid June and early August and this was the case with the samples selected for this study.

Table 2 indicates the range of samples taken with respect to harvest cut, and ensiling method. This data indicates that there was an approximately equal split between baled and clamp silages and that approximately 75% of silages were first cut with the remainder being second

cut. This is considered to be an accurate representation of the proportions of these types of silages found on sheep and beef farms across Wales.

**Table 2 - Breakdown of silages analysed**

	Number in sample set	% of total
All Silages	58	100
Clamp Silages	27	46.55
Baled Silages	31	53.45
First Cut Silages	45	77.59
Second Cut Silages	13	22.41
First Cut Clamp	23	39.66
Second Cut Clamp	4	6.70
First Cut Bale	22	37.93
Second Cut Bale	9	15.52

As already stated it is important to get a range of different samples. Tables 3, 4 and 5 show the mean and ranges for major silage components as predicted by the NIR analyses for all, clamp and bale silages respectively analysed in this project.

**Table 3 – Mean (and range) silage analyses for all 58 silages sampled**

	Mean	Maximum	Minimum
DM (g/kg FM)	412.20	860.60	207.90
D value (%)	68.23	78.40	54.57
pH	4.61	5.63	3.50
Crude Protein (g/kg DM)	134.82	194.80	81.66
NDF (g/kg DM)	521.93	641.14	371.59
ADF (g/kg DM)	348.58	593.73	184.15
ME (mJ/Kg)	10.92	12.54	8.73
Ash (g/kg DM)	77.98	106.71	46.45
WSC (g/kg DM)	58.37	202.84	2.01
Lactic acid (g/kg DM)	66.26	187.78	7.55
Acetic Acid (g/kg DM)	18.02	56.41	5.00
Butyric Acid (g/kg DM)	6.18	23.66	3.00
Ammonia-N (% TN)	9.69	28.40	1.08

**Table 4 – Mean (and range) silage analyses for all 27 clamp silages sampled**

	Mean	Maximum	Minimum
DM (g/kg FM)	343.56	638.62	207.90
D value (%)	67.58	75.94	54.97
pH	4.29	5.13	3.50
Crude Protein (g/kg DM)	137.91	194.80	91.82
NDF (g/kg DM)	528.59	635.74	382.10
ADF (g/kg DM)	325.12	546.02	184.15
ME (mJ/Kg)	10.81	12.15	8.73
Ash (g/kg DM)	75.98	106.71	46.45
WSC (g/kg DM)	36.60	116.38	2.01
Lactic acid (g/kg DM)	87.90	187.78	19.26
Acetic Acid (g/kg DM)	23.87	56.41	5.00
Butyric Acid (g/kg DM)	6.76	23.66	3.00
Ammonia-N (% TN)	9.04	25.19	1.10

**Table 5 – Mean (and range) silage analyses for all baled 31 silages sampled**

	Mean	Maximum	Minimum
DM (g/kg FM)	471.98	860.60	210.43
D value (%)	68.79	78.40	56.82
pH	4.90	5.63	4.08
Crude Protein (g/kg DM)	132.12	175.02	81.66
NDF (g/kg DM)	516.13	641.14	371.59
ADF (g/kg DM)	369.01	593.73	270.48
ME (mJ/Kg)	11.01	12.54	9.09
Ash (g/kg DM)	79.72	105.14	50.90
WSC (g/kg DM)	77.34	202.84	3.62
Lactic acid (g/kg DM)	47.42	112.23	7.55
Acetic Acid (g/kg DM)	12.92	49.21	5.00
Butyric Acid (g/kg DM)	5.67	20.26	3.00
Ammonia-N (% TN)	10.25	28.40	1.08

The data in tables 3 – 5 indicate that the project successfully sampled a wide range of samples and that the range was wide for both baled and clamped silages. If for example we examine the DM content of silages analysed. This ranged from 20 – 64 % in clamps with a mean of 34% and 21 – 86 % in bales with a mean of 47%. As DM is probably the biggest factor affecting many of the other chemical components and particularly fermentation parameters the project was successful in providing a wide range of silages to test the analytical procedures. Variations in Crude Protein (CP) and Metabolizable Energy (ME) contents were also suitably wide with 8.1 – 19.4 % CP and 8.7 to 12.4 mJ/kg DM ME when looking at the whole sample set. With similarly wide variations for silages from bales and clamps a like.

As a whole the data from the silages sampled using the mean NIR analyses indicates that the baled silage quality was superior to that of clamp with 0.2 mj.kg DM more ME with similar protein levels. Compared to the national average figures for 2010 released by Frank Wright (urgent news: No 144: Grass Silage Update - 2010/2011; issues 4/10/10) the silage quality of

this group across all silages was better in ME by 0.1 MJ/kg DM and similar for CP. The results are very encouraging in terms of mean silage quality on Welsh sheep and beef farms competing with, what in effect will be silages predominantly from the UK dairy sector, as reported by Frank Wright. The underlying range of silage quality shows that on many farms there is still a lot of potential improvement that is required to maximise the utilisation of forage on these farms and therefore on profitability.

### Comparison of Results provided by Wet Chemical and NIR analyses

Table 6 shows the predicted means and effects of analysis type on silage composition as analysed using standard wet chemistry analysis methods compared with NIR prediction. Of the analyses reported, significant differences in mean composition were observed for pH, crude protein and lactic acid. NIR analysis (as indicated in the effect column) under-predicted pH by an average of 0.48 units, and under-predicted CP concentrations by an average of 22 g/kg DM. Lactic acid concentrations, on the other hand, were over-predicted by NIR by an average of 15 g/kg DM. No significant differences in mean DM contents, ammonia-N or NDF concentrations were found.

Table 6. Predicted means and effects of analysis method for all silages in the study (n=58).

	Analysis method		SED	Effect	P
	Wet Chemistry	NIR			
Dry matter, g/kg	422.4	412.2	9.11	-10.3	0.265
pH	5.09	4.61	0.091	-0.48	<0.001
Crude protein, g/kg DM	157.1	134.8	3.75	-22.3	<0.001
Ammonia-N, g/kg DM	8.47	9.69	0.764	1.22	0.116
Lactic acid, g/kg DM	51.2	66.3	3.90	15.1	<0.001
Neutral detergent fibre, g/kg DM	508.0	521.9	8.96	14.0	0.125

Table 7 shows the predicted means and effects of analysis type on baled silages only (of both first and second cuts). Among baled silages, NIR tended ( $P = 1.07$ ) to under-predict DM content compared with standard drying methods. On the other hand, although CP concentrations were under-predicted, lactic acid and pH values were not significantly different between wet chemistry and NIR techniques.

Table 7. Predicted means and effects of analysis method for baled silages only (of both cuts) (n=27).

	Analysis method		SED	Effect	P
	Wet Chemistry	NIR			
Dry matter, g/kg	364.4	343.6	12.47	-20.9	0.107
pH	4.37	4.29	0.088	-0.08	0.348
Crude protein, g/kg DM	152.7	137.9	5.67	-14.8	0.015
Ammonia-N, g/kg DM	9.81	9.05	1.333	-0.77	0.571
Lactic acid, g/kg DM	80.3	87.9	6.89	7.6	0.281
Neutral detergent fibre, g/kg DM	519.0	528.6	13.92	9.6	0.496

The major (significant) differences in analytical values seen among all silages was attributed to the subset of clamped silages of both cuts (Table 8) and to first cut silages of both types (Table 9). Perhaps surprisingly Table 8 indicates that NIR predictions were significantly different from wet chemical analysis in all analytes with the exception of DM and NDF. Out of all comparisons the expectation was that the NIR prediction for clamped silages would have been the most accurate.

Table 8. Predicted means and effects of analysis method for clamped silages only (of both cuts) (n=31).

	Analysis method		SED	Effect	P
	Wet Chemistry	NIR			
Dry matter, g/kg	473.0	472.0	13.10	-1.0	0.938
pH	5.72	4.90	0.122	-0.82	<0.001
Crude protein, g/kg DM	160.9	132.1	4.75	-28.8	<0.001
Ammonia-N, g/kg DM	7.30	10.25	0.722	2.95	<0.001
Lactic acid, g/kg DM	25.8	47.4	3.92	21.6	<0.001
Neutral detergent fibre, g/kg DM	498.4	516.1	11.74	17.8	0.141

Table 9 shows the comparison between NIR prediction and wet chemistry for all first cut silages. Again there are significant differences in values for pH, crude protein and lactic acid. Next to clamp silages, the assumption would be that first cut silages would have had a better prediction than latter cuts. However again this seems not to be the case. There is of course the fact that many of the first cut silages in this sample set will be at a time of year more typical of second cut silages from many regions of England, so this may be another contributory factor to the inaccuracy of the NIR methodology due to the silages used in the calibration being unrepresentative of all UK silages.

Table 9. Predicted means and effects of analysis method for first cut silages only (of both silage types) (n=45).

	Analysis method		SED	Effect	P
	Wet Chemistry	NIR			
Dry matter, g/kg	428.6	411.0	11.0	-17.6	0.115
pH	5.08	4.58	0.106	-0.50	<0.001
Crude protein, g/kg DM	153.4	133.9	4.04	-19.43	<0.001
Ammonia-N, g/kg DM	8.41	9.41	0.856	1.01	0.247
Lactic acid, g/kg DM	53.8	68.9	4.54	16.1	<0.001
Neutral detergent fibre, g/kg DM	514.7	530.3	10.15	15.7	0.132

Table 10 shows the comparison between NIR prediction and wet chemistry for all second cut silages. This analysis is generally better than those highlighted previously but there is still a significant under prediction of the crude protein content by NIR of 31g/kg DM.

Table 10. Predicted means and effects of analysis method for second cut silages only (of both silage types) (n=13).

	Analysis method		SED	Effect	P
	Wet Chemistry	NIR			
Dry matter, g/kg	401.2	416.5	12.70	15.3	0.250
pH	5.13	4.73	0.174	-0.40	0.040
Crude protein, g/kg DM	170.0	137.9	8.95	-31.1	0.004
Ammonia-N, g/kg DM	8.67	10.64	1.74	1.97	0.280
Lactic acid, g/kg DM	45.5	57.0	7.73	11.5	0.163
Neutral detergent fibre, g/kg DM	484.7	493.1	19.70	8.4	0.678

## Conclusions

The results shown in this report indicate that there is a significant problem with the use of NIR prediction of silage composition, particularly in the case of Crude Protein which across all silages has been underestimated by 22 g/kg DM. There are also significant differences, depending on silage selection pool (ie, clamp or bale, first or second cut), with all the analyses except NDF which at times is very close to being significantly different. For these reasons, it is somewhat of an arbitrary exercise to calculate the costs associated with these poor analyses as so much must be done to rectify the situation. So if we just take one scenario and use the crude protein difference for the mean value for all silages between wet and NIR analysis of 22g/kg DM. In a beef finishing animal assuming 8 kg silage DM intake that represents 176g of crude protein not being accounted for from the silage. If that protein was to be replaced by rape seed meal (38% protein and costing £200/tonne) there would be an increased supplementation of 463g/d taking that over a 150 day winter feeding period would mean an extra 70 kg rapeseed meal/animal at a cost of £14/animal and so on a 100 animal unit £1,400.

The bottom line is that there are many different hypothetical scenarios and what the industry needs is a reliable silage analysis that enables the best judgements to be made in winter rationing for all grades of stock on the farm.

It is important that farm silage analysis should be continued to be done, but for Crude Protein a wet chemical analysis should be conducted. On sheep and beef farms the other analyses are important but in terms of rationing it is the Crude Protein figure that is the most important.

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