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Environmental and Nutritional Benefits of Bioethanol Co-products (ENBBIO LINK)

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Environmental and nutritional benefits of bioethanol co-products

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1. Abstract

The ENBBIO LINK project was devised to bring together all sectors of the bioethanol, livestock and arable industries to investigate the value of wheat DDGS produced by the growing bioethanol industry, for all sectors of UK livestock production. The non-ruminant programme was designed to examine the nutritional value of wheat distillers dark grains with solubles (W-DDGS) in poultry and pigs. Nine separate trials were undertaken based on a range of objectives / methodologies. A large-scale commercial broiler trial (H2S) revealed that there were no differences in liveweight, but better Feed Conversion Ratio with W-DDGS although these diets were more expensive as a result of having to include higher levels of pure amino acids; however cost /kg gain was lower and Production Efficiency Factor (PEF) higher. The trial has shown that the addition of up to 100g/kg W-DDGS into a balanced broiler diet, had no detrimental effects on the technical performance of the birds. An initial layer trial (Nottingham) reported that including W-DDGS at up to 180 g/kg in diets that were isoenergetic and balanced for digestible amino acids had no effect on performance and egg shell quality; there were no effects of treatment on gut environment / microflora. The next commercial layer trial (Noble) reported that, with an inclusion of 75 g/kg W-DDGS with the nutritional matrix values ascribed to the raw material in the formulations by Premier Nutrition, there was no practical difference between the trial and control flocks. W-DDGS can be safely used in layer diets, in part substituting for imported soya. Whether it is actually used or not will depend on the relative values of the product and other raw materials used in least cost formulated layer diets. Growing / finishing pigs are able to tolerate levels of W-DDGS up to 300g/kg in pelleted balanced diets in terms of performance and carcass quality without a significant reduction in performance. In a final commercial growth trial (Tulip, Harper Adams), the inclusion of Wheat Dried Distillers Grains (W-DDGS) at any of the levels in the pelleted diets did not have any negative effects for on farm performance, slaughter characteristics or meat quality. The highest inclusion at 300 g/kg showed best performance in a number of areas including daily liveweight gain, FCR and slaughter weight. It can therefore be concluded that feeding pigs during the growing and finishing stages with up to 300 g/kg W-DDGS included in the pelleted diets is an acceptable level.

The ENBBIO ruminant studies achieved their primary objective, which was to evaluate Wheat DDGS (wDDGS) from UK bioethanol production in terms of nutritional value and animal responses to inclusion in typical ruminant diets. The first dairy trial gave an apparent limitation of wDDGS inclusion of ~200 g/kg of diet dry matter. Digestibility studies confirmed that there was no significant effect of wDDGS inclusion level on dry matter digestibility. A second dairy trial re-examined the effect of inclusion level of wDDGS. For this trial, diets were formulated with ME values and degradation characteristics determined in advance for the actual batch of wDDGS to be tested. With an accurate ME value, there was no effect of wDDGS inclusion level on intake or

performance. In a survey of wDDGS use on commercial beef farms, inclusion levels of 125 g/kg and 300 g/kg of the diet supported good performance levels.

An important element of the ENBBIO project was to quantify the potential environmental benefits of bioethanol production, focussing on the utilisation of the co-products and their value in the animal feed supply chain. Using 3 Mt of wheat grown on 405 kha of UK arable land to produce DDGS, in addition to the bioethanol produced, would potentially substitute for ca. 1Mt of three major commodities used in animal feeds i.e. SBM, SFM and wheat. The extent to which of DDGS will substitute for other commodities, particularly plant proteins, will inevitably show some variation over time, for instance as economic scenarios and the relative prices of different feed ingredients change. An estimated 389 kt of SBM could be substituted, which equates to 150 kha land area spared.

Another element of work within the ENBBIO project was to identify and create options by which industries producing DDGS might enhance the quality and value of their DDGS. A general recommendation is that this will happen most effectively in the context of integrated approaches that allow additional product revenue streams and more efficient operation. A long-term view in which DDGS is produced in increasingly integrated biorefineries will provide helpful guidance and direction for the development of the industry. The opportunity to extract arabinoxylan is particularly promising that would enhance the nutritional quality and commercial scope of DDGS while producing an additional high value product. The project demonstrated great value for wDDGS in its current form as well as potential for further improvements.

2. Introduction

The Environmental and Nutritional Benefits of Bioethanol Co-Products (ENBBIO LINK) project started on 1st October 2010 and ended in December 2014. The Government sponsor was Defra, through Sustainable Livestock Production LINK programme. The aim of this research was to quantify sources of variability in wheat distillers grains and solubles (W- DDGS), identify opportunities to enhance their value, to consider innovative processes to reduce fibre content (for non-ruminants) and to quantify the contribution of the co-products to the overall GHG balance of UK crop, livestock and ethanol production.

3. Poultry nutritional and performance studies

3.1. Nottingham Broiler 1

Title: The determination of (1) performance (Feed intake, feed conversion ratio, liveweight gain) (2) ileal Nitrogen/AA digestibility and (3) Nitrogen retention of diets based on varying levels of Wheat Distillers Dark Grains with Solubles (W-DDGS).

3.1.1. Summary and conclusions

a. No significant differences were detected between treatments in terms of performance during both starter and grower phases; no starter x grower interactions were obtained indicating that feeding W-DDGS during the starter phase did not lead to any adaptation during the grower phase.

When performance was assessed over the entire trial (data for starter and grower were combined), there was evidence that birds fed 5% W-DDGS in the starter experienced an inferior FCR overall with increasing W-DDGS in the grower.

Comments received from commercial colleagues during initial reporting were that, although differences were not, generally, statistically significant, numerical changes between treatments would be of some considerable importance in a production context. This is a common observation when attempting to reconcile statistical with commercial validity of data.

Data (with error terms and P values) are presented in full in the results sections; but are summarised below:

i. Starter (0-14d)

	DDGS %	
	0%	5%
LWG g	930	930
FI g	1174	1160
FCR	1.26	1.25

ii. Grower (14-27d)

	LWG g/cage				FI g/cage				FCR cage			
DDGS Starter	DDGS % Grower				DDGS % Grower				DDGS % Grower			
	0%	6%	12%	18%	0%	6%	12%	18%	0%	6%	12%	18%
0%	2102	1970	1980	2069	3096	3002	3008	3013	1.47	1.53	1.52	1.47
5%	2074	2039	1962	1995	3054	3045	3002	3089	1.47	1.51	1.53	1.56

iii. Overall

DDGS Starter	LWG g/cage				FI g/cage				FCR cage			
	DDGS % Grower				DDGS % Grower				DDGS % Grower			
	0%	6%	12%	18%	0%	6%	12%	18%	0%	6%	12%	18%
0%	3032	2927	2905	2993	4313	4193	4133	4156	1.42	1.43	1.42	1.39
5%	3012	3044	2899	2934	4254	4234	4187	4257	1.41	1.39	1.44	1.45

b. A reduction in the coefficient of apparent ileal nitrogen and amino acid digestibility was observed with increasing levels of W-DDGS. SID values for the W-DDGS studied may need to be lowered.

i. Nitrogen

Starter	DDGS % Grower			
	0%	6%	12%	18%
0%	0.89	0.784	0.762	0.698
5%	0.88	0.785	0.751	0.766

ii. Selected amino acids

DDGS Starter	LYS				MET + CYS				THR			
	DDGS % Grower				DDGS % Grower				DDGS % Grower			
	0%	6%	12%	18%	0%	6%	12%	18%	0%	6%	12%	18%
0%	0.884	0.816	0.838	0.813	0.871	0.788	0.799	0.767	0.808	0.691	0.725	0.681
5%	0.884	0.83	0.837	0.847	0.872	0.804	0.799	0.811	0.809	0.718	0.731	0.74

c. In view of uncertainties over amino acid digestibility (the current trial had used 'assumed' values as directed by Defra LINK), this was examined in a subsequent trial.

3.1.2. Trial Objectives and Basic Design

The current trial is one of the initial 'production' trials designed to examine performance of broilers fed diets containing graded levels of W-DDGS in isocaloric and isonitrogenous diets balanced for standardised ileal digestible amino acids with all diets containing exogenous phytase.

The study was conducted with Ross 308 males housed in the Poultry Metabolism Unit at the University of Nottingham.

<u>Starter (0-14d)</u>

32 cages on each of two treatments (+/- W-DDGS)

- Performance (liveweight gain, feed intake, feed conversion ratio on a CAGE basis; with each cage containing two birds) measured over 14 days.

- Precise measurements of feed intake and excreta output over the last three days to allow N balance to be calculated.

Grower (14-27d)

16 cages (eight fed W-DDGS in Starter and eight fed Control without DDGS in Starter) on each of four treatments; this approach was selected to examine whether or not prior feeding of a diet with W-DDGS would influence subsequent performance.

- Performance (liveweight gain, feed intake, feed conversion ratio on a CAGE basis; with each cage containing two birds) measured over 14 days.
- Precise measurements of feed intake and excreta output over the last three days to allow N balance to be calculated.
- Ileal digesta samples removed at slaughter on day 28 sent to Evonik for amino acid analysis ileal digestibility determined with reference to the inert marker acid insoluble ash.

The statistical models used were a simple two treatment for the starter and a 2 (starter) x 4 (grower) factorial, with linear and non-linear contrasts to account for the incremental increases in W-DDGS, for the grower phase.

In both cases, initial liveweight was used as a covariate when analysing for performance. Two birds per cage are crucial for balance studies so, in the event of any mortality, the dead bird was removed and weighed to be replaced immediately by a spare weighed bird fed the same diet. Following discussion it was agreed that, in the event of a replacement bird being introduced, that cage would be treated as a missing value with one cage in the starter and nine in the grower (mortality not linked to diet).

Both analyses of variance include cage position as a factor; no significant differences were detected.

3.1.3. Test Diets

Two experimental starter (0 and 5% W-DDGS) and four experimental grower diets were prepared by Target Feeds based on formulations prepared by AB Vista; two grower extremes (0 and 18% W-DDGS) were initially manufactured and then blended to give two intermediary diets (6 and 12% W-DDGS). Details are in Appendix 1. Diets were fed as mash.

3.1.4. Results

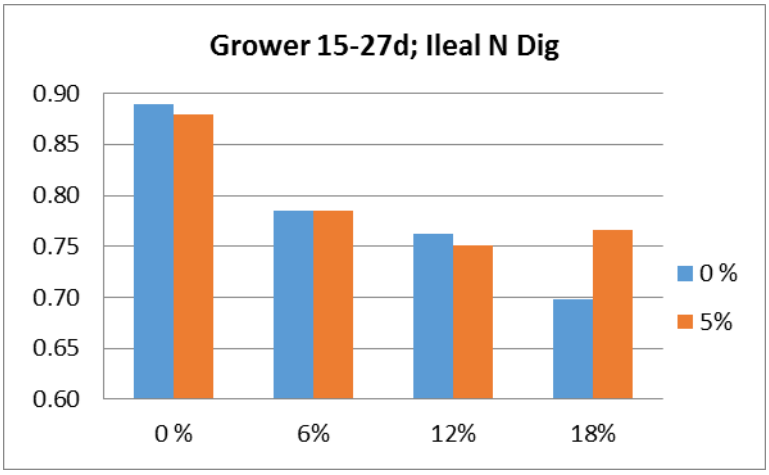
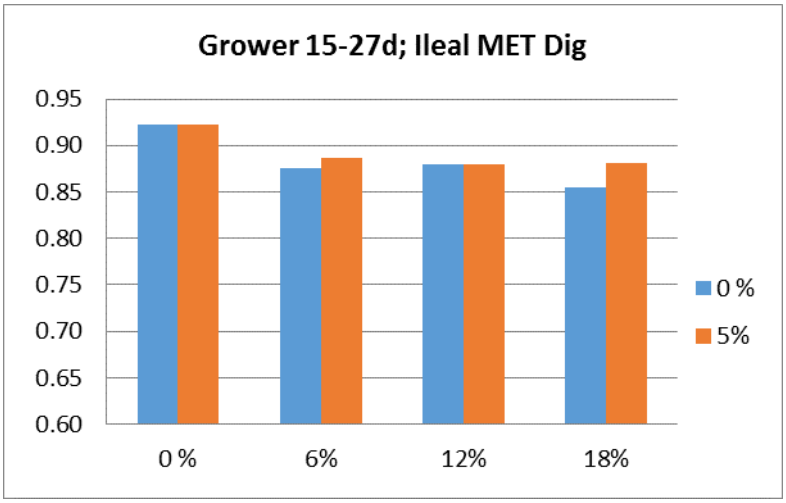


Figure 1 Summary response coefficient of ileal N digestibility

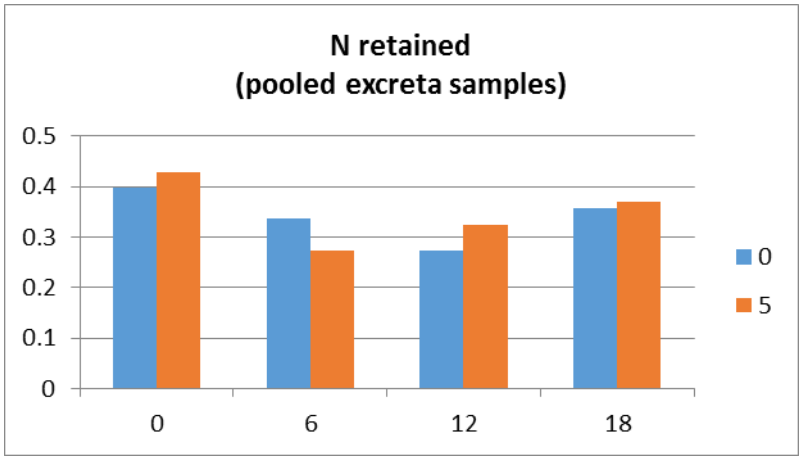


Data represent pooled ileal digesta samples (8 cages per column). Digesta was pooled as requested, to yield sufficient material for laboratory analyses

Figure 2 Summary response coefficient of ileal MET Digestibility (as an example of an amino acid)

Data for the coefficient of total tract apparent N retention revealed a significant reduction in the starter phase at 5% W-DDGS inclusion compared with the control. There were no carryover effects of the starter into the grower phase and no starter * grower interactions. However there was a significant quadratic relationship between W-DDGS inclusion and N retention; highest values were obtained with 0% W-DDGS followed by a reduction with evidence then of a small recovery at 18% (but not to the level obtained with the 0% W-DDGS diet).

When data were expressed subsequently as content of retained nitrogen (coefficient of total tract apparent N retention x dietary nitrogen), these overall responses did not change.



Data represent pooled excreta samples (8 cages per column). Excreta pooled as per ileal digesta for comparable analysis.

Figure 3 Summary response coefficient of total tract apparent N retention

In conclusion broilers are able to tolerate levels of W-DDGS up to 18% in terms of performance; there was no evidence of any carryover effects between starter and grower in terms of acclimatisation to W-DDGS in the former phase although, when overall performance was considered, there was a poorer response in those birds fed W-DDGS in the starter.

Despite there being no differences in terms of performance, there was clear evidence of a reduction in both nitrogen and amino acid digestibility and nitrogen retention with increasing levels of W-DDGS suggesting that the diet formulation exercise had over-estimated SID amino acid values. The lack of correlation between performance and digestibility / retention is problematic.

3.1.5. Results (data and statistical analyses)

Performance: all data are per CAGE

Table 1 Liveweights at specific days

Diet and % DDGS		Liveweight at d			Diet and % DDGS		Liveweight at d		
Starter	Grower	1	14	27	Starter	Grower	1	14	27
0	0	83.8	907.2	2963.6	5	0	84.4	926.9	2866.2
5	6	93.3	1095.8	3353.8	0	6	89.8	1040.0	3344.5
0	12	75.9	985.6	2997.1	5	12	82.4	998.4	2944.9
5	18	74	1115.9	2690.0	0	18	76.4	946.5	2920.3
0	0	82	1021.6	2986.5	5	0	83.4	1082.2	3285.3
5	6	81.1	1011.2	2784.6	0	6	95.2	1084.9	3458.4
0	12	100.9	1049.7	3034.2	5	12	80.7	969.6	3180.1
5	18	80	1018.5	3018.9	0	18	76.8	1090.9	2876.4
5	0	87.7	1091.1	3179.9	0	0	100.2	1073.3	3221.3
0	6	89.1	1052.6	3224.0	5	6	88.4	1022.7	3277.4
5	12	94.9	1027.9	2904.5	0	12	100.2	1051.5	3277.1
0	18	70.1	996.5	2897.1	5	18	84.2	989.7	3133.1
5	0	78.4	1025.1	3182.0	0	0	91.1	1051.2	3149.2
0	6	83.6	928.8	2640.7	5	6	123.3	1112.3	2604.9
5	12	84.6	1037.4	2831.7	0	12	76.9	999.3	3060.8
0	18	89.5	1016.0	3437.7	5	18	87.5	927.7	3031.1
0	0	74.5	1019.1	3109.3	5	0	91.7	986.6	3018.1
5	6	87.4	1005.0	3098.6	0	6	79.2	1011.8	2576.2
0	12	84.3	1052.3	2875.9	5	12	82.4	1088.9	3201.8
5	18	94.2	1047.6	3088.4	0	18	84.2	1032.5	3238.5
0	0	91.5	1072.5	3185.8	5	0	83.5	1010.3	3076.5
5	6	90.7	1072.4	3054.7	0	6	84.7	1024.0	3158.8
0	12	82.7	944.0	2785.9	5	12	80.5	1011.6	2935.2
5	18	98.9	1102.5	3135.2	0	18	100.3	964.2	3026.1
5	0	69.2	859.0	2902.8	0	0	88.4	974.5	3045.0
0	6	80.5	1058.2	3260.1	5	6	85.2	1011.7	3075.7
5	12	94.5	1018.6	3054.5	0	12	84.4	918.6	3019.0
0	18	96.2	991.9	3052.1	5	18	84.2	1047.0	3164.2
5	0	87.6	993.1	3028.5	0	0	91.1	1121.8	3395.1
0	6	75.2	982.9	3015.4	5	6	89.4	878.4	2950.0
5	12	84.1	984.6	3027.2	0	12	81.5	989.2	2857.6
0	18	78.4	1022	2993.9	5	18	89.6	1021.4	2973.6

Shaded cells: missing value in ANOVA for performance

	Starter
	Grower

Table 2 Starter (0-14d); liveweight gain (LWG g), feed intake (g), feed conversion ratio (FCR)

	DDGS %		CV%	SED	P=
	0%	5%			
LWG	930	930	5.9	13.6	0.986
FI	1174	1160	5.8	17	0.412
FCR	1.26	1.25	3.4	0.011	0.145

Table 3 Grower (14-27d) liveweight gain (LWG g)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	2102	1970	1980	2069	2030
5%	2074	2039	1962	1995	2017
Mean	2088	2004	1971	2032	2024
	Factor		CV%	SED	P=
	Starter		9.1	45.9	0.775
	Grower			64.6	0.327
					0.326 L
					0.120 Q
					0.827 D
	Starter x Grower			91.5	0.732
					0.578 L
					0.407 Q
					0.600 D

Table 4 Grower (14-27d) feed intake (g)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	3096	3002	3008	3013	3030
5%	3054	3045	3002	3089	3047
Mean	3075	3024	3005	3051	3039
	Factor		CV%	SED	P=
	Starter		6.8	51.8	0.755
	Grower			72.9	0.792
					0.698 L
					0.355 Q
					0.894 D
	Starter x Grower			103.2	0.857
					0.515 L
					0.894 Q
					0.566 D

Table 5 Grower (14-27d) feed conversion ratio (g)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	1.47	1.53	1.52	1.47	1.5
5%	1.47	1.51	1.53	1.56	1.52
Mean	1.47	1.52	1.53	1.51	1.51
	Factor		CV%	SED	P=
	Starter		5.8	0.022	0.360
	Grower			0.031	0.312
					0.166 L
					0.200 Q
					0.954 D
	Starter x Grower			0.044	0.273
					0.127 L
					0.214 Q
					0.935 D

No significant differences were detected; in the tables L = linear, Q = quadratic, D = deviations from quadratic.

Table 6 Overall liveweight gain (LWG g)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	3032	2927	2905	2993	2964
5%	3012	3044	2899	2934	2972
Mean	3022	2985	2902	2963	2968
	Factor		CV%	SED	P=
	Starter		5.6	42	0.884
	Grower			59.1	0.240
					0.168 L
					0.254 Q
					0.308 D
	Starter x Grower			84	0.503
					0.508 L
					0.278 Q
					0.369 D

Table 7 Overall feed intake (FI g)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	4313	4193	4133	4156	4199
5%	4254	4234	4187	4257	4233
Mean	4284	4214	4160	4206	4216
	Factor		CV%	SED	P=
	Starter		5.5	58.3	0.575
	Grower			82	0.512
					0.272 L
					0.324 Q
					0.745 D
	Starter x Grower			116.6	0.803
					0.348 L
					0.829 Q
					0.825 D

Table 8 Overall feed conversion ratio (FCR)

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	1.42	1.43	1.42	1.39	1.42
5%	1.41	1.39	1.44	1.45	1.43
Mean	1.42	1.41	1.43	1.42	1.42
	Factor		CV%	SED	P=
	Starter		4.0	0.014	0.575
	Grower			0.02	0.706
					0.518 L
					0.877 Q
					0.333 D
	Starter x Grower			0.029	0.081
					0.033 L
					0.219 Q
					0.386 D

There was a significant starter x grower interaction (0.033 L) with a deterioration in birds fed 5% DDGS in the starter phase experiencing a deterioration in FCR overall with increasing DDGS in the grower**.**

N and amino acid balance

Table 9 Coefficient of apparent ileal nitrogen digestibility determined at the end of the grower phase

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	0.890	0.784	0.762	0.698	0.784
5%	0.880	0.785	0.751	0.766	0.796
Mean	0.885	0.784	0.757	0.732	0.790
	Factor		CV%	SED	P=
	Starter		4.6	0.0171	0.505
	Grower			0.0241	0.001
					<0.001 L
					0.056 Q
					0.395 D
	Starter x Grower			0.0341	0.364
					0.179 L
					0.353 Q
					0.492 Q

No significant effect of starter diet was observed, so no carryover effects into the grower and no starter * grower interaction. There was a significant linear reduction (P<0.001) in data and a very strong trend for this to be quadratic (P=0.056).

Table 10 Amino acid content (%) of diets supplied by Evonik; data based on DM contents shown (diets were oven dried before being sent to Evonik)

	Starter		Grower			
	0	5	0	6	12	18
DM	98.57	98.50	98.86	98.63	98.62	98.51
MET	0.64	0.66	0.55	0.54	0.56	0.54
CYS	0.42	0.39	0.39	0.39	0.37	0.37
M+C	1.06	1.06	0.94	0.92	0.93	0.91
LYS	1.56	1.47	1.29	1.29	1.29	1.33
THR	1.03	0.97	0.85	0.87	0.85	0.88
ARG	1.74	1.68	1.35	1.44	1.45	1.54
ILE	1.12	1.05	0.92	0.95	0.94	0.98
LEU	1.93	1.85	1.64	1.69	1.65	1.70
VAL	1.22	1.15	1.04	1.07	1.04	1.07
HIS	0.64	0.61	0.52	0.55	0.54	0.56
PHE	1.30	1.24	1.11	1.14	1.11	1.14
GLY	1.10	1.04	0.95	0.97	0.94	0.96
SER	1.28	1.22	1.10	1.12	1.10	1.12
PRO	1.65	1.54	1.68	1.65	1.51	1.46
ALA	1.10	1.05	0.93	0.96	0.94	0.97
ASP	2.57	2.49	1.93	2.07	2.12	2.27
GLU	5.34	4.99	5.11	5.06	4.76	4.67
MET free	0.29	0.30	0.22	0.21	0.20	0.21
LYS free	0.24	0.18	0.33	0.27	0.21	0.17
THR free	0.07	0.06	0.04	0.04	0.04	0.04

Table 11 Coefficient of apparent ileal amino acid digestibility determined at the end of the grower phase

A. Methionine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.923	0.876	0.880	0.855	0.884
5%	0.922	0.887	0.880	0.881	0.893
Mean	0.923	0.882	0.880	0.868	0.888
	Factor		CV%	SED	P=
	Starter		1.6	0.0069	0.217
	Grower			0.0097	0.003
					<.001 L
					0.067 Q
					0.146 D
	Starter x Grower			0.0138	0.504
					0.306 L
					0.603 Q
					0.333 D

The results of the analysis of variance is very close to that observed for N ileal digestibility; similar results were obtained for all other amino acids.

B. Cystine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.796	0.662	0.696	0.649	0.701
5%	0.795	0.679	0.693	0.711	0.720
Mean	0.796	0.671	0.695	0.680	0.710
	Factor		CV%	SED	P=
	Starter		5.2	0.0184	0.338
	Grower			0.0261	0.005
					0.005 L
					0.017 Q
					0.052 D
	Starter x Grower			0.0369	0.600
					0.340 L
					0.548 Q
					0.478 D

C. Methionine + cystine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.871	0.788	0.799	0.767	0.806
5%	0.872	0.804	0.799	0.811	0.822
Mean	0.872	0.796	0.799	0.789	0.814
	Factor		CV%	SED	P=
	Starter		3.0	0.0121	0.245
	Grower			0.0170	0.004
					0.002 L
					0.027 Q
					0.130 D
	Starter x Grower			0.0241	0.582
					0.336 L
					0.583 Q
					0.427 D

D. Lysine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.884	0.816	0.838	0.813	0.838
5%	0.884	0.83	0.837	0.847	0.850
Mean	0.884	0.823	0.838	0.830	0.844
	Factor		CV%	SED	P=
	Starter		2.3	0.0097	0.267
	Grower			0.0137	0.008
					0.01 L
					0.024 Q
					0.057 D
	Starter x Grower			0.0194	0.580
					0.341 L
					0.619 Q
					0.398 D

E. Threonine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.808	0.691	0.725	0.681	0.726
5%	0.809	0.718	0.731	0.74	0.750
Mean	0.809	0.705	0.728	0.711	0.738
	Factor		CV%	SED	P=
	Starter		4.4	0.0161	0.188
	Grower			0.0228	0.006
					0.006 L
					0.028 Q
					0.047 D
	Starter x Grower			0.0323	0.587
					0.316 L
					0.684 Q
					0.418 D

F. Arginine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.885	0.805	0.828	0.795	0.828
5%	0.885	0.821	0.827	0.837	0.843
Mean	0.885	0.813	0.828	0.816	0.835
	Factor		CV%	SED	P=
	Starter		2.3	0.0096	0.183
	Grower			0.0136	0.002
					0.002 L
					0.014 Q
					0.031 D
	Starter x Grower			0.0193	0.406
					0.234 L
					0.501 Q
					0.319 D

G. Isoleucine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.849	0.758	0.787	0.744	0.785
5%	0.852	0.781	0.791	0.786	0.803
Mean	0.851	0.770	0.789	0.765	0.794
	Factor		CV%	SED	P=
	Starter		3.2	0.0127	0.197
	Grower			0.0180	0.005
					0.003 L
					0.057 Q
					0.035 D
	Starter x Grower			0.0255	0.702
					0.425 L
					0.734 Q
					0.452 D

H. Leucine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.852	0.768	0.8	0.765	0.796
5%	0.853	0.789	0.803	0.803	0.812
Mean	0.853	0.779	0.802	0.784	0.804
	Factor		CV%	SED	P=
	Starter		3.0	0.012	0.225
	Grower			0.0170	0.009
					0.009 L
					0.046 Q
					0.036 D
	Starter x Grower			0.0241	0.688
					0.415 L
					0.776 Q
					0.427 D

I. Valine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.833	0.726	0.757	0.712	0.757
5%	0.834	0.75	0.764	0.76	0.777
Mean	0.834	0.738	0.761	0.736	0.767
	Factor		CV%	SED	P=
	Starter		3.8	0.0146	0.206
	Grower			0.0206	0.005
					0.003 L
					0.040 Q
					0.035 D
	Starter x Grower			0.0291	0.662
					0.356 L
					0.755 Q
					0.466 D

J. Histidine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.86	0.767	0.787	0.745	0.790
5%	0.86	0.773	0.792	0.792	0.804
Mean	0.860	0.770	0.790	0.769	0.797
	Factor		CV%	SED	P=
	Starter		3.1	0.0125	0.283
	Grower			0.0176	0.003
					0.002 L
					0.026 Q
					0.028 D
	Starter x Grower			0.0249	0.538
					0.241 L
					0.484 Q
					0.652 D

K. Phenylalanine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.858	0.783	0.816	0.792	0.812
5%	0.856	0.801	0.819	0.826	0.826
Mean	0.857	0.792	0.818	0.809	0.819
	Factor		CV%	SED	P=
	Starter		2.6	0.0107	0.249
	Grower			0.0151	0.015
					0.038 L
					0.030 Q
					0.031 D
	Starter x Grower			0.0214	0.637
					0.354 L
					0.777 Q
					0.420 D

L. Tryptophan

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.852	0.746	0.771	0.725	0.774
5%	0.846	0.769	0.776	0.785	0.794
Mean	0.849	0.758	0.774	0.755	0.784
	Factor		CV%	SED	P=
	Starter		3.3	0.0128	0.144
	Grower			0.0181	0.003
					0.002 L
					0.022 Q
					0.039 D
	Starter x Grower			0.0256	0.351
					0.162 L
					0.611 Q
					0.318 D

M. Glycine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.82	0.699	0.727	0.674	0.730
5%	0.819	0.719	0.73	0.736	0.751
Mean	0.820	0.709	0.729	0.705	0.741
	Factor		CV%	SED	P=
	Starter		4.5	0.0165	0.234
	Grower			0.0234	0.004
					0.002 L
					0.030 Q
					0.048 D
	Starter x Grower			0.0331	0.557
					0.281 L
					0.592 Q
					0.470 D

N. Serine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.834	0.744	0.763	0.722	0.766
5%	0.834	0.758	0.768	0.775	0.784
Mean	0.834	0.751	0.766	0.749	0.775
	Factor		CV%	SED	P=
	Starter		3.4	0.013	0.202
	Grower			0.0184	0.005
					0.003 L
					0.035 Q
					0.059 D
	Starter x Grower			0.026	0.516
					0.246 L
					0.538 Q
					0.504 D

O. Proline

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.867	0.79	0.833	0.806	0.824
5%	0.868	0.812	0.833	0.84	0.838
Mean	0.868	0.801	0.833	0.823	0.831
	Factor		CV%	SED	P=
	Starter		2.5	0.0103	0.196
	Grower			0.0146	0.012
					0.058 L
					0.026 Q
					0.016 D
	Starter x Grower			0.0206	0.603
					0.419 L
					0.746 Q
					0.321 D

P. Alanine

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.832	0.722	0.747	0.694	0.749
5%	0.834	0.743	0.751	0.748	0.769
Mean	0.833	0.733	0.749	0.721	0.759
	Factor		CV%	SED	P=
	Starter		4.2	0.0161	0.243
	Grower			0.0227	0.005
					0.002 L
					0.055 Q
					0.056 D
	Starter x Grower			0.0322	0.647
					0.362 L
					0.633 Q
					0.480 D

Q. Aspartic acid

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.814	0.707	0.735	0.685	0.735
5%	0.814	0.732	0.74	0.741	0.757
Mean	0.814	0.720	0.738	0.713	0.746
	Factor		CV%	SED	P=
	Starter		3.9	0.0144	0.178
	Grower			0.0204	0.004
					0.002 L
					0.042 Q
					0.042 D
	Starter x Grower			0.0289	0.532
					0.280 L
					0.650 Q
					0.394 D

R. Glutamic acid

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.888	0.825	0.854	0.83	0.849
5%	0.887	0.843	0.853	0.861	0.861
Mean	0.888	0.834	0.854	0.846	0.855
	Factor		CV%	SED	P=
	Starter		2.2	0.0093	0.244
	Grower			0.0131	0.018
					0.033 L
					0.040 Q
					0.043 D
	Starter x Grower			0.0185	0.560
					0.368 L
					0.725 Q
					0.313 D

Table 12 Coefficient of apparent nitrogen retention (CANR)

A. Starter

	DDGS %		CV%	SED	P=
	0%	5%			
CANR	0.376	0.330	23.8	0.0211	0.033

There was a significant reduction in N retention when birds were fed a diet containing 5% W-DDGS.

B. Grower

Starter	DDGS % Grower				Mean
	0%	6%	12%	18%	
0%	0.399	0.336	0.274	0.356	0.341
5%	0.429	0.273	0.323	0.369	0.348
Mean	0.414	0.304	0.298	0.362	0.345
	Factor		CV%	SED	P=
	Starter		18.7	0.0323	0.836
	Grower			0.0457	0.106
					0.297L
					0.023 Q
					0.823 D
	Starter x Grower			0.0646	0.648
					0.833L
					0.667 Q
					0.259 D

There was no significant effect of starter on N retention during the grower phase. There was a significant quadratic relationship between W-DDGS inclusion and N retention; highest values were obtained with 0% W-DDGS followed by a reduction with evidence then (P=0.023Q) of a recovery at 18% (but not to the level obtained with the 0% W-DDGS diet). No starter * grower interactions were recorded.

Table 13 Content of apparent retained nitrogen (ARN, g/kg DM)

Data are calculated from those in table 8 multiplied by total dietary content of N

A. **Starter**

Total (g/kg DM)

DDGS %	
0%	5%
36.3	36.6

	DDGS %				
	0%	5%	CV%	SED	P=
ARN	14	12	23.9	0.8	0.046

B. **Grower**

Total (g/kg DM)

DDGS % Grower			
0 %	6%	12%	18%
32.9	30.2	30.8	31.9

Starter	DDGS % Grower				Mean
	0 %	6%	12%	18%	
0 %	13	10	8	11	11
5%	14	8	10	12	11
Mean	14	9	9	12	11
	Factor		CV%	SED	P=
	Starter		18.3	1.0	0.815
	Grower			1.4	0.037
					0.195 L
					0.009 Q
					0.661 D
	Starter x Grower			2.0	0.652
					0.849 L
					0.660 Q
					0.262 D

Although there were small differences in total dietary N content, these did not have an important effect; thus responses are very similar to those in Table 8.

3.2. Nottingham Broiler 2

Title: The determination of (1) Standardised ileal Nitrogen/AA digestibility and (2) Nitrogen retention of broilers offered diets based on Wheat Distillers Dark Grains with Solubles (W-DDGS).

3.2.1. Summary and conclusions

a. Values for apparent ileal digestibility (AID) and standard ileal digestibility (SID) of amino acids were similar to those reported elsewhere in the literature, although SID values for lysine were particularly low, being 0.26, 0.27 or 0.32, measured in semi-synthetic, maize or wheat diet backgrounds, respectively.

b. It appeared that diet type employed was influential in the values obtained. The SID values for methionine, cysteine, methionine plus cysteine and arginine were significantly lower ($P < 0.05$) when measured in semi-synthetic diet backgrounds than wheat or corn-based diets.

c. It does appear that dextrose and possibly purified starch have a detrimental impact on the broiler digestive tract. This may impact upon all digestibility methodologies where such a diet base is used.

Data (with error terms and P values) are presented in full in the results sections; but are summarised below:

	Diet types		
	SS	Corn	Wheat
Lysine	0.26	0.27	0.32
Methionine	0.64	0.7	0.71
Cystine	0.52	0.65	0.68
Methionine + Cystine	0.58	0.68	0.69
Threonine	0.56	0.56	0.58
Isoleucine	0.62	0.62	0.63
Leucine	0.66	0.68	0.68
Valine	0.52	0.56	0.54
Histidine	0.6	0.59	0.61
Phenylalanine	0.73	0.74	0.74
Arginine	0.58	0.68	0.69

3.2.2. Trial Objectives and Basic Design

The current trial was designed to supplement data in Nottingham Broiler 1 where data suggested that text-book SID values for W-DDGS were an overestimate.

There has been considerable debate in the ENBBIO non-ruminant sub-group as to the nature of the diets to be used. As a result it has been agreed that the trial would be based on five diets A-E: Diet A = semi-synthetic with W-DDGS as the only proteinaceous raw material, Diet B was Maize-based without W-DDGS, Diet C was Maize-based with W-DDGS, Diet D was Wheat-based without W-DDGS and Diet E was Wheat-based with W-DDGS inclusion where SID amino acids would be determined by difference.

Each experimental diet was fed (in mash form) to eight replicates of a cage containing two Ross 308 broilers from day 21; collection of excreta and ileal digesta allowed calculation of Nitrogen retention and AID / SID data.

Test Diets

	Diet (g/kg)				
	A	B	C	D	E
Maize		660	295		
Wheat				660	295
Soya 48		245	110	245	110
DDGS	500		500		500
Starch	205				
Glucose	200				
Oil	50	50	50	50	50
Premix	40	40	40	40	40
Ti02	5	5	5	5	5
TOTAL	**1000**	**1000**	**1000**	**1000**	**1000**

1. Results

Table 14 Content of standard ileal digestible AA g/kg diet DM

	Diet (g/kg)				
	A	B	C	D	E
Lys	0.79	9.11	4.97	9.29	5.22
Met	1.71	2.56	3.02	2.67	3.07
Thr	3.43	5.94	6.05	6.13	6.27
Ile	3.92	6.23	6.72	7.27	7.23
Val	4.21	6.61	7.51	7.45	7.79
Leu	8.65	14.82	15.53	13.54	14.96
His	2.19	4.39	4.11	4.71	4.33
Phe	6.56	8.14	10.32	9.16	10.82
Arg	4.45	11.39	9.71	11.98	10.08
Cys	3.45	3.70	5.95	5.03	6.76
Met + Cys	5.41	6.66	9.30	7.96	10.01

Table 15 Content of standardised ileal digestible amino acids in W-DDGS g/kg DM as influenced by basal diet

	Synthetic	Maize	Wheat
Lys	1.579	1.783	2.132
Met	3.430	3.746	3.757
Thr	6.867	6.781	7.059
Ile	7.833	7.856	7.961
Val	8.424	9.114	8.904
Leu	17.308	17.789	17.801
His	4.371	4.286	4.442
Phe	13.116	13.348	13.432
Arg	8.905	9.230	9.435
Cys	6.895	8.592	9.022
Met + Cys	10.829	12.640	12.903

Data for Maize and Wheat calculated by difference; data for B divided by 405/905 to reflect rate of inclusion of protein components in Diet C and then deducted from data from Diet C giving contribution of W-DDGS @500g/kg; latter data multiplied by 2 to provide data @ 1000 g W-DDGS/kg.

Table 16 Total amino acids in W-DDGS g/kg DM

	TOTAL
Lys	6.68
Met	5.32
Thr	12.18
Ile	12.64
Val	16.36
Leu	26.18
His	7.28
Phe	18.06
Arg	14.66
Cys	13.30
Met + Cys	18.62

Table 17 The coefficient of standardised ileal digestibility (SID) of amino acid in wheat DDGS measured broilers affected by diet type

	Diet types							
	Semi-synthetic[1]		Corn[2]		Wheat[2]			
	Mean	SD	Mean	SD	Mean	SD	P	RMSE
Lysine	0.26	0.102	0.27	0.036	0.32	0.039	0.056	0.064
Methionine	0.64b	0.046	0.70a	0.012	0.71a	0.039	0.004	0.035
Cystine	0.52b	0.058	0.65a	0.049	0.68a	0.049	<0.001	0.057
Methionine + Cystine	0.58b	0.048	0.68a	0.029	0.69a	0.049	<0.001	0.043
Threonine	0.56	0.058	0.56	0.022	0.58	0.024	0.463	0.037
Isoleucine	0.62	0.061	0.62	0.023	0.63	0.031	0.871	0.040
Leucine	0.66	0.038	0.68	0.021	0.68	0.023	0.357	0.028
Valine	0.52	0.073	0.56	0.027	0.54	0.037	0.250	0.048
Histidine	0.6	0.057	0.59	0.022	0.61	0.029	0.548	0.038
Phenylalanine	0.73	0.049	0.74	0.014	0.74	0.014	0.491	0.029
Arginine	0.58b	0.042	0.68a	0.018	0.69a	0.022	<0.001	0.043

[a,b] Within a row, means without common superscripts are significantly different as indicated by the P value.

[1]Using direct method; [2]Using the difference method.

Table 18 The coefficient of apparent ileal digestibility (AID) of amino acids of the experimental diets measured in broilers

	Dietary treatments[1]						
Amino acids	S-DDGS	C	C-DDGS	W	W-DDGS	P	RMSE
Lysine	0.16c	0.82a	0.58b	0.79a	0.59b	<0.001	0.059
Methionine	0.61c	0.85a	0.74b	0.83a	0.74b	<0.001	0.067
Cystine	0.49b	0.59ab	0.62ab	0.70a	0.68a	0.040	0.130
Methionine + Cystine	0.55b	0.72a	0.68a	0.76a	0.71a	0.003	0.095
Threonine	0.47c	0.68a	0.57b	0.68a	0.58b	<0.001	0.048
Isoleucine	0.56c	0.76a	0.65b	0.77a	0.66b	<0.001	0.058
Leucine	0.63c	0.79a	0.71b	0.78a	0.71b	<0.001	0.043
Valine	0.46c	0.69a	0.58b	0.69a	0.57b	<0.001	0.065
Histidine	0.54c	0.76a	0.64b	0.77a	0.66b	<0.001	0.048
Phenylalanine	0.70d	0.79ab	0.74c	0.80a	0.75bc	<0.001	0.036
Arginine	0.58d	0.85a	0.72c	0.81b	0.71c	<0.001	0.035

[1]S-DDGS, semisynthetic diet containing DDGS; C, corn diet; C-WDDGS, corn diet containing DDGS; W, wheat diet; W-DDGS, wheat diet containing DDGS.

[a-d] Within a row, means without common superscripts are significantly different as indicated by the P value.

Table 19 The content of standardised ileal digestible amino acids in each diet (g/kg)

	Dietary treatments[1]				
Amino acids	S-DDGS	C	C-DDGS	W	W-DDGS
Lysine	0.79	9.11	4.97	9.29	5.22
Methionine	1.71	2.56	3.02	2.67	3.07
Threonine	3.43	5.94	6.05	6.13	6.27
Isoleucine	3.92	6.23	6.72	7.27	7.23
Valine	4.21	6.61	7.51	7.45	7.79
Leucine	8.65	14.82	15.53	13.54	14.96
Histidine	2.19	4.39	4.11	4.71	4.33
Phenylalanine	6.56	8.14	10.32	9.16	10.82
Arginine	4.45	11.39	9.71	11.98	10.08
Cystine	3.45	3.70	5.95	5.03	6.76
Methionine + Cystine	5.41	6.66	9.30	7.96	10.01

[1]S-DDGS, semisynthetic diet containing DDGS; C, corn diet; C-WDDGS, corn diet containing DDGS; W, wheat diet; W-DDGS, wheat diet containing DDGS.

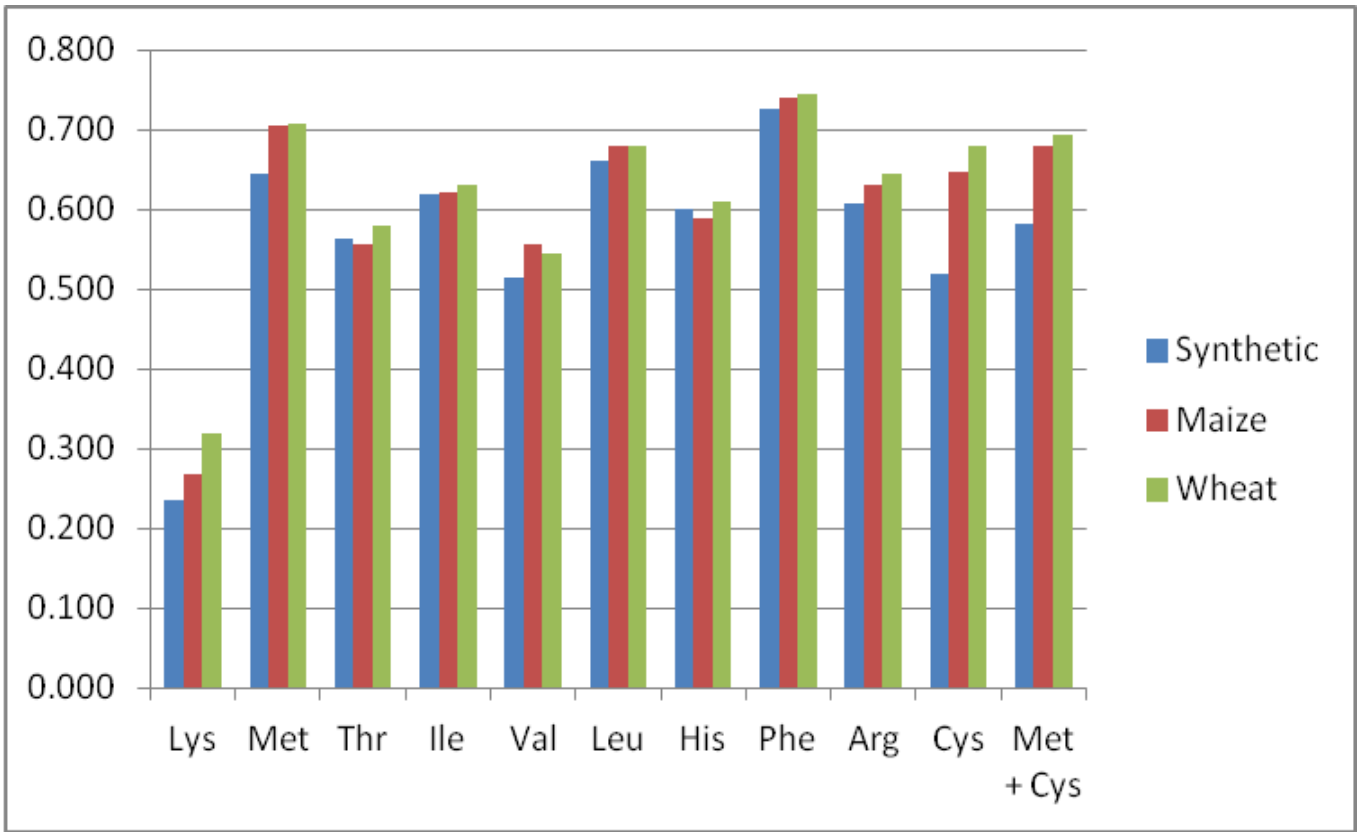


Figure 4 Coefficient of standardised ileal digestible amino acids in W-DDGS as influenced by basal diet (data from Table 5).

3.3. H2S Commercial Broiler Trial 1

Title: The determination of performance in a commercial setting of birds fed diets based on W-DDGS.

3.3.1. Summary

- No differences in liveweight.
- Better Feed Conversion Ratio with W-DDGS.
- W-DDGS-based diets were more expensive as a result of having to include higher levels of pure amino acids; however cost /kg gain was lower and Production Efficiency Factor (PEF) higher.
- Hock marking and pododermatitis were lower in W-DDGS-based diets.

It is difficult to draw absolute conclusions from 1 commercial trial, however the trial has shown that the addition of up to 10% W-DDGS into a pelleted balanced broiler diet, had no detrimental effects on the technical performance of the birds. The concerns of the effects that W-DDGS may have on litter quality were not shown in the trial work. More commercial trials need to be carried out to further back up this initial work. The findings did suggest that levels of W-DDGS up to a level of

10%, combined with the use of additional Amino Acids, could provide an alternative protein source in broiler diets.

Trial objectives and basic design

Studies show W-DDGS is able to be a valuable raw material for poultry if it can compete economically with other protein sources such as sunflower meal, rapeseed meal and wheatfeed in least cost formulations. Whilst these studies have also shown that up to 18% W-DDGS can be used safely in both broiler and layer diets and in part as a substitute for imported soya, there is still concern within the poultry industry over the variability of W-DDGS and its low bulk density. It is vital that the quality and consistency of the material in terms of its digestible nutrient content in known and that this information is fully utilised in the diet formulation process if maximal levels are to be used in the diet.

Two houses of Ross 708 as hatched broilers were placed on the same commercial broiler site. The flocks were mirrored with a total of 37,290 broilers placed in each house. Both of the houses used were identical in terms of construction and equipment used and are typical of a modern broiler site within the UK. House 1 was used as the trial house, with house 4 used as the control.

The site was fully disinfected and fumigated prior to the commencement of the trial and the birds were placed on a clean bedding mixture 60%/40% chopped straw and shavings. Any top up bedding used was also of the same mixture.

Both houses used the same temperature targets and minimum ventilation rates, lighting programmes was 1 hour dark to 7 days, with 6 hours (spilt 4 and 2) to 24 hours prior to depletion. The birds were grown to a target weight of 1.90kg, when approximately 33% were removed. The remainder were grown to a weight of 2.90kg and were then all depleted. The following data was recorded:

- Mortality (%)
- Feed Intake
- Water Intake
- Average Age
- Average weight
- Food Conversion Ratio (FCR)
- Production Efficiency Factor (PEF)
- Pododermatitis (%)
- Hock Marking (%)
- Reject (%)
- Additional Litter use

- Litter Score:
    1. surface completely dry and friable (breaks apart when pressed in hand)
    2. slightly moist (does not break apart when pressed in hand)
    3. moist/capped under drinkers
    4. capped but now dry
    5. capped and wet
    6. wet and soggy
- Feed cost p/kg

3.3.2. Test Diets

A standard feed was used in the control house (Table 20 and Table 21). The trial diet had the addition of 10% W-DDGS which replaced a percentage of the soya. The diets were formulated to the same energy and digestible lysine levels (determined in Nottingham Broiler 2). Both diets contained the same amount of whole wheat and used the same coccidiostats as can be seen in Table 21. Both Trial and control houses were fed ad libitum. Feed intake was recorded individually via the use of a tipper weighing system for each diet.

Table 20 Diets

	Starter Crumb	Starter Pellet	Grower Pellet	Finisher Pellet	Withdrawal Pellet
Wheat	40.448	41.53	47.3725	53.829	53.106
Treated Wheat	-	5	7.5	0	5
Maize	5	5	5.09	5	5.04
Maize Germ	2.9	4	0	5	2.4
Wheat DDG & Syrup	10	10	10.1	7.5	7.5
Soya Hipro	29.3	24	20.2	18.2	17.2
Rapeseed	1	1.5	1.9	2	1.9
Pulse/Rape Blend	5	3	2.3	2	1.9
Broiler B Premix	-	-	-	0.25	0.2375
Broiler Str/Gwr Premix	-	0.25	0.23125	-	-
H2S Broiler Str/Gwr Premix	0.25	-	-	-	-
L-Lysine	0.542	0.48	0.501	0.485	0.467
DL-Methionine	0.336	0.274	0.243	0.225	0.212
Threonine	0.096	0.056	0.076	0.068	0.065
Actigen	0.04	0.04	0.037	0.04	0.038
Betaine HCl	0.066	0.066	0.061	0.066	0.063
Ronozyme ProAct	0.02	0.02	0.0185	0.02	0.019
Q Blue 5L (Liquid) Stock Code	0.01	0.01	0.00925	0.01	0.0095
Econase	0.01	0.01	0.00925	0.01	0.0095
Lysoforte	0.05	0.05	0.04625	0.05	0.0475
Monteban G100	-	-	-	0.07	0.07
Nicarb/Koffogran (Nicarbazin 25%)	0.05	-	-	-	-
Limestone	0.77	0.83	0.66	0.75	0.73
DCP Aliphos	1.04	0.75	0.85	0.64	0.4
Rock Salt	0.172	0.134	0.095	0.087	0.086
Soya Oil	2.9	3	2.7	3.7	3.5

Table 21 Diet make up

Diet	Wheat %	ME	Dig lysine	Coccidiostat
Starter Crumb 0-10 days	0	12.9	1.31	Nicarbazin
Starter pellet 11-18 days	5	13.1	1.13	Maxiban
Grower pellet 19-24 days	7.5	13.1	1.1	Maxiban
Finisher pellet 25-31 days	0	13.57	0.98	Monteban
Withdrawal pellet 32-end	0	13.57	0.98	Monteban

3.3.3. Results

The houses were placed as follows in Table 22 with flocks mirrored from the same hatchery source. Both houses followed the same vaccination programme, given both at day old at the hatchery and on farm.

Table 22 Placement Details

House Number	**House 1**	**House 4**
Flock Code	RUS 40	RUS 40
Number Placed	34176	34176
Flock code	R1 43	R1 43
Number placed	3114	3114
Total Placed	**37,290**	**37,290**

Mortality was broken down by in terms of actual mortality and culls and as can be seen from Figure 5 there was no significant difference between the trial and control house, with the trial recording 3.52% and the control 3.26%.

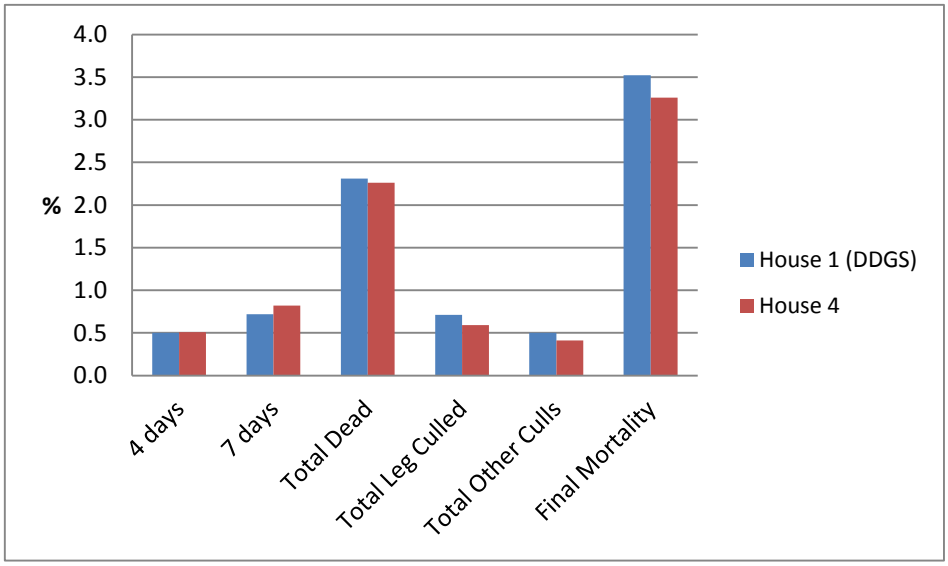


Figure 5 Mortality Breakdown

Birds were weighed on a weekly basis, with a random sample of approximately 100 birds taken from each house. The final weight recorded was taken from factory data and adjusted to age due to house 1 being depleted over 2 days (46 and 47 days), whilst house 4 was depleted at 47 days. It was assumed a growth rate of 90 grams with the following calculation used:
Adjusted weight = ((47- actual age) x 0.09) +actual weight

Figure 6 shows the weight performance measured against the breed target as a percentage. As can be seen both houses were ahead of breed target up to 21 days, with both houses achieving breed target at 42 days and no significant difference at depletion.

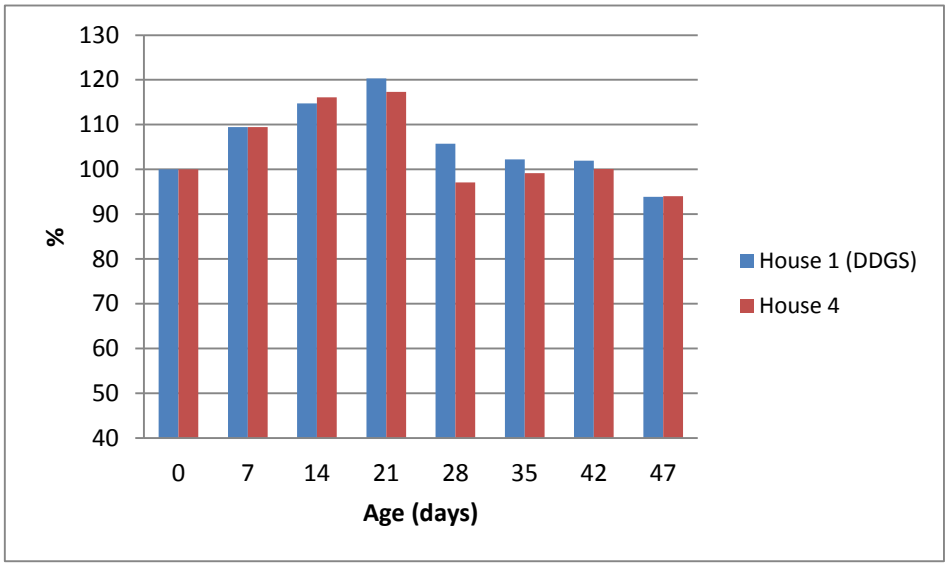


Figure 6 Weight Performance Against Breed Target (%)

Table 23 shows the combined weights and ages from both the thin and final depletions. Although the age of thin and a proportion of the final depletions were different, when calculated back to an average of 42 days, there was no significant difference.

Table 23 Summary of weights and ages

House Number	House 1 W-DDGS	House 4
Thinned age	33.00	35.00
Thinned weight	1.86	2.00
Clear Age	46.68	47.00
Depletion weight	2.91	2.95
Average Age	42.02	42.85
Average weight	2.55	2.62
Weight for age (42 days)	2.55	2.54

There was a difference in average diet cost of £5.07 per tonne, with the W-DDGS diet being more expensive. The reasons for this difference, was that additional protein in the form of pure amino acids had to be forced into the diet in order to achieve the required digestible lysine levels.

Food Conversion Ratio (FCR) was calculated taking the feed consumed per house divided by the total weight sent to the processing plant. This included any dead on arrival and reject figures. FCR was then adjusted back to 42 days, assuming a movement in FCR of 0.02 points per day as per the breed specification. The following calculation was used:

Adjusted FCR = Actual FCR – ((42 – actual age) x 0.02)

As can be seen from Table 24 there was a significant difference in FCR, with the house 1 showing a 0.20 point advantaged in adjusted FCR. Despite the difference in diet cost this gave an advantage in terms of feed cost per kg of live-weight of 4 pence per bird.

Table 24 Feed and Technical Results

House Number	House 1 W-DDGS	House 4
FCR	1.55	1.73
Adjusted FCR (42 days)	1.55	1.75
Feed cost per kg live weight (£)	0.43	0.47
Water to feed ratio	1.99	1.91
PEF	377	342

The difference in FCR when combined with the other performance factors, gave a significant difference in Production Efficiency Factor (PEF) of 35 points. PEF was calculated as follows:

PEF = (liveability x live weight kg)/(Age in days x FCR)

The amount of additional litter was recorded, along with the litter score for each house. As can be seen from Table 25 there was no significant difference between the 2 houses.

Table 25 Comparison of Additional Litter and Litter Scores

House	**1** **W-DDGS**	**4**
Total Top up bales	247	227
Highest Litter Score	2	2

Both hock marking and pododermatitis was recorded at the processing plant, with a sample of 100 birds taken from each load and recorded as a %. Values were recorded as presence or absence. As can be seen from Figure 7, there was a difference with the trial house recording lower levels for both hock marking and pododermatitis.

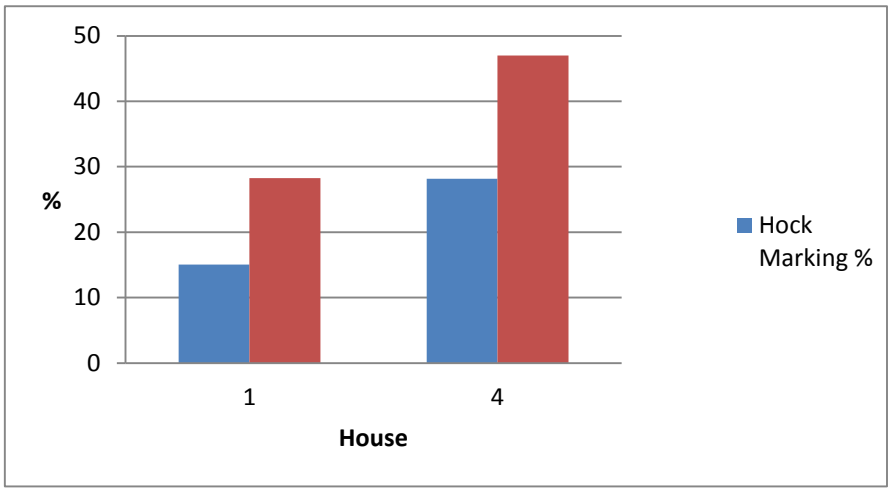


Figure 7 Comparisons of Hock Marking and Pododermatitis

3.4. Nottingham Layer 1

Title**:** The determination of (1) Performance (Feed intake, feed conversion ratio, egg output / quality) (2) Nitrogen retention (3) Gut Health of layers offered diets based on varying levels of Wheat Distillers Dark Grains with Solubles (W-DDGS).

3.4.1. Summary and conclusions

a. Including W-DDGS at up to 18% in diets that were isoenergetic and balanced for digestible amino acids had no effect on performance and egg shell quality, on the basis of which a commercial evaluation trial was then planned by Noble Foods.

b. There were no effects of treatment on gut environment / microflora.

Key data are presented below

	Diet			
	D0	D60	D120	D180
Caecal parameters[1]				
Caecal pH	7.3	7.0	6.3	6.1
SCFA (mmol/L)				
Acetic	78	88	88	89
Propionic	30	34	35	37
Butyric	14	19	20	23
Iso-Butyric	3.8	2.7	3.2	2.0
Valeric	9.6	9.1	11.6	10.2
Total SCFA	135.4	152.8	157.8	161.2
Performance parameters				
Feed Intake (g/day)	112	112	110	116
CAM_N[2]	0.399	0.349	0.391	0.361
Egg production [3]				
g egg/hen/day	49	49	49	48

[1] Data collected from birds at slaughter (at 31 weeks of age)

[2] Coefficient of Apparent Metabolisability of Nitrogen

[3] Data from weeks 2, 3 and 4 of the trial

3.4.2. Trial objectives and basic procedures

The current trial was one of the 'production' trials designed to examine performance of layers fed diets containing graded levels of W-DDGS in isocaloric and isonitrogenous diets balanced for standard ileal digestible amino acids with all diets containing exogenous Finase P5000 and Econase. Performance was assessed over four weeks, early in lay starting from week 27.

The hypothesis was that increasing the level of DDGS in layer diets that are isocaloric and balanced for standard ileal digestible amino acids would not influence performance, digestibility or gut health.

A total collection of excreta was undertaken over 3 days mid-trial.

At the end of the trial, birds were slaughtered and caecal contents collected for gut environment (caecal pH, short-chain fatty acids) and bacteriological assessment (lactobacillus, clostridia, enterobacteriacae, bifidobacterium).

Test diets

These are presented in Table 26 (fed as mash).

3.4.3. Results

Birds took time to adjust to their new surroundings and experimental diets; accordingly data from the initial week were not analysed.

There was no effect or treatment on feed intake (Table 26), egg weight (Table 27) or gut environment (Table 28). Diversity profiles of the bacterial 16S rRNA gene from luminal caecal contents were unaffected by W-DDGS inclusion.

Other than performance, egg quality was also examined for dirty shells. All eggs laid were photographed and sent to Noble Foods for an opinion. A representative sample of specific days is presented in Figure 8 Noble Foods were of the opinion that W-DDGS included at up to 18% of the diet had no detrimental effect on egg shell quality.

Table 26 Diets and assumed composition (%, except ME MJ/kg)

Ingredient	0% DDGS W	18% DDGS G
Wheat - Feed	58.76	52.78
DDGS		18.00
Corn Glutenmeal 60	4.00	4.00
Soybean meal 48	14.27	5.40
Sunflower meal	7.50	4.00
Soy oil	4.05	4.47
Salt	0.20	0.10

Sodium Bicarbonate	0.20	0.03
DL Methionine	0.09	0.11
Lysine HCl	0.16	0.40
Limestone	9.11	9.31
Dicalcium Phos	1.15	0.90
Mono Na Phos	0.006	0.00%
Finase P 5000	0.006	0.006
Vitamin premix	0.49	0.49
Econase XT	0.0075	0.0075
Crude protein	18.24	18.27
Poult ME MJ/kg	11.72	11.72
Calcium	3.90 (4.02)	3.90 (4.02)
Phos	0.68	0.63
Avail Phos	0.40 (0.53)	0.40 (0.53)
Fat	5.56	6.89
Fibre	3.65	3.87
Met	0.40	0.40
Cys	0.33	0.34
Me+Cys	0.73	0.74
Lys	0.86	0.86
His	0.44	0.44
Tryp	0.21	0.20
Thr	0.63	0.60
Arg	1.08	0.85
Iso	0.72	0.70
Leu	1.52	1.47
Phe	0.88	0.88
Tyr	0.62	0.60
Val	0.82	0.83
Gly	0.77	0.77
Ser	0.92	0.91
Phe+Tyr	1.51	1.48
D Met	0.36	0.36
D Cys	0.30	0.31
D Me+Cys	0.66	0.67
D Lys	0.77	0.77

D His	0.40	0.39
D Tryp	0.19	0.18
D Thr	0.57	0.54
D Arg	0.97	0.76
D Iso	0.64	0.63
D Leu	1.37	1.33
D Val	0.74	0.74
D Gly	0.69	0.69
D Ser	0.83	0.82
Phytate P	0.20	0.18
Na	0.17	0.17
Cl	0.20	0.21
K	0.61	0.57
Linoleic acid	2.24	2.68
Na+K-Cl	171.52	160.66
DUA	1,838.98	1,855.64
Sulphur	0.20	0.18
Magnesium	0.12	0.15
Betaine	0.77	0.71
Choline	1224.05	1,161.37
Gly+ser	1.68	1.68

Diets were blended in the appropriate proportions to give intermediary diets 6 (P) and 12% (B) W-DDGS.

Table 27 Feed intake (weeks 3-4); initial liveweight as a covariate

a. Total (g)

Diet (% W-DDGS)			
0	6	12	18
1688	1634	1582	1690

	P	
Diet	0.115	SED 49.3, CV 5.9%
Lin	0.109	
Quad	0.436	
Covariate	0.884	

b. Daily (g)

Diet (% W-DDGS)			
0	6	12	18
121	117	113	121

	P	
Diet	0.115	SED 3.5, CV 3.5 %
Lin	0.109	
Quad	0.436	
Covariate	0.884	

Table 28 Laying performance

a. % lay

	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	**Mean**
Week 2	68.8	81.3	81.3	84.4	87.5	87.5	93.8	**83.5**
Week 3	96.9	97.0	100.0	100.0	93.8	96.9	100.0	**97.8**
Week 4	96.9	93.8	100.0	90.6	84.4	87.5	100.0	**93.3**
Mean weeks 2, 3 and 4								**91.5**

b. Mean egg weight (weeks 3 and 4)

Diet (% W-DDGS)			
0	6	12	18
56	53	54	56

	P	
Diet	0.220	SED 1.4, CV 5.4%
Lin	0.589	
Quad	0.396	

Table 29 Effect of increasing level of Wheat Distillers Dried Grains with Solubles on caecal and performance parameters of layer hens (from 27-31 weeks of age)

	Diet					*P*		
	D0	D60	D120	D180	Sed	Diet	Linear	Quadratic
Caecal parameters[1]								
Caecal pH	7.3	7.0	6.3	6.1	0.28	<0.001	<0.001	0.202
SCFA (mmol/L)								
Acetic	78	88	88	89	9.9	0.647	0.313	0.451
Propionic	30	34	35	37	5.6	0.638	0.301	0.452
Butyric	14	19	20	23	3.9	0.130	0.074	0.138
Iso-Butyric	3.8	2.7	3.2	2.0	0.73	0.115	0.319	0.036
Valeric	9.6	9.1	11.6	10.2	2.01	0.626	0.268	0.506
Total SCFA	135.4	152.8	157.8	161.2	19.38	0.534	0.210	0.451
Performance parameters								
Feed Intake (g/day)	112	112	110	116	3.5	0.353	0.927	0.215
CAM_N[2]	0.399	0.349	0.391	0.361	0.0438	0.631	0.926	0.209
Egg production [3]								
g egg/hen/day	49	49	49	48	2.9	0.967	0.945	0.766

[1] Data collected from birds at slaughter (at 31 weeks of age)

[2] Coefficient of Apparent Metabolisability of Nitrogen

[3] Data from weeks 2, 3 and 4 of the trial

D0, D60, D120 and D180 represent diets containing 0, 60, 120 and 180g W-DDGS/kg respectively.

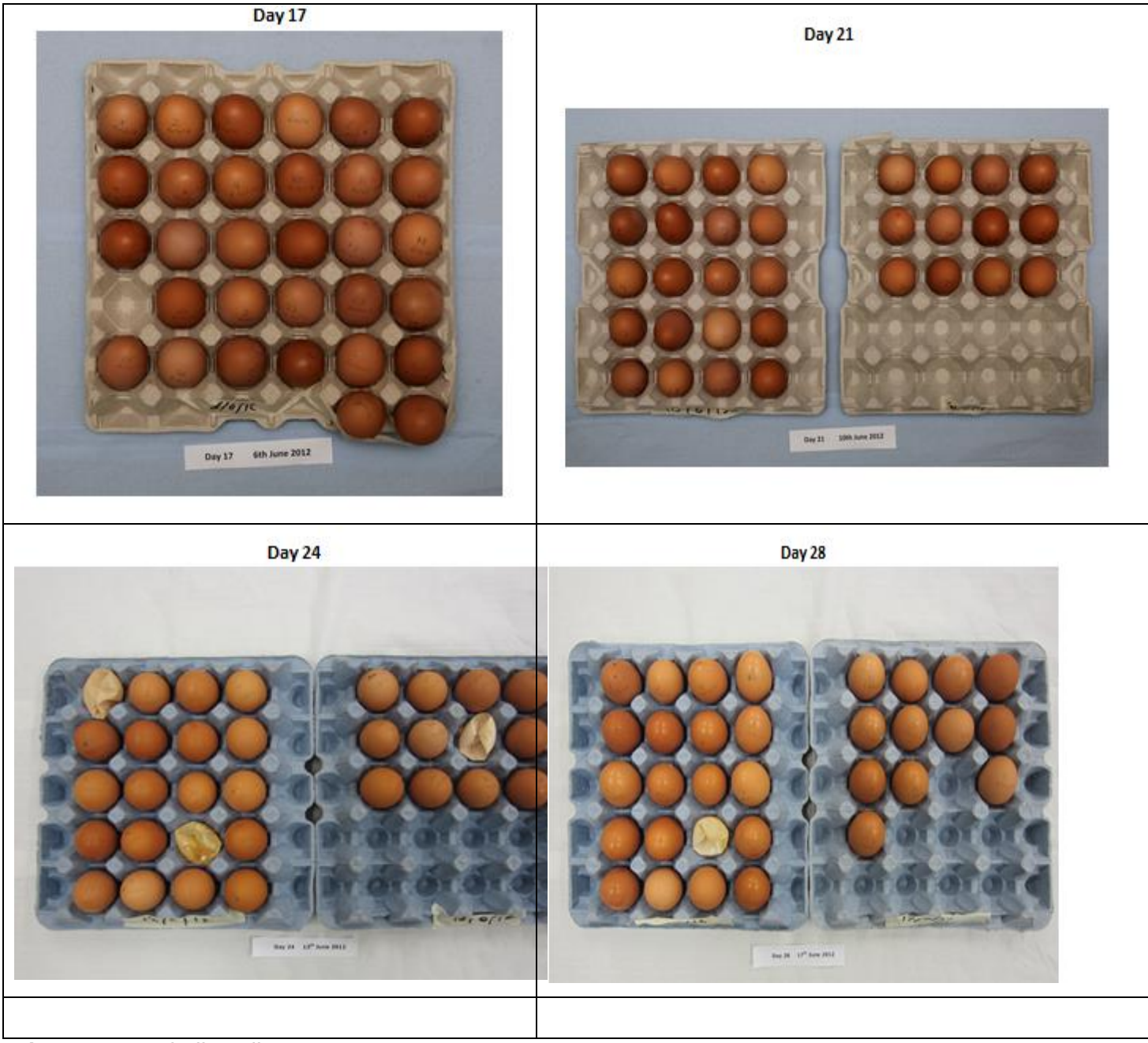


Figure 8 Egg shell quality

3.5. Noble Layer

Title: Determination of performance and egg quality in commercial layer flocks fed diets containing W-DDGS.

3.5.1. Summary and conclusions

a. With an inclusion of 7.5% WDDGS, with the nutritional matrix values ascribed to the raw material in the formulations by Premier Nutrition, there was no practical difference between the trial and control flocks. In addition and in particular concerns over potential increased seconds from using WDDGS were not realised.

b. WDDGS therefore can be safely used in layer diets, in part substituting for imported soya. Whether it is actually used or not will depend on the relative values of the product and other raw materials used in least cost formulated layer diets. However, at recent market values it would not feature in a typical layer diet.

c. The WDDGS was supplied as produced at the plant in a non-pelleted format. In this state the material has a low bulk density which made transport costs excessive due to very poor load factors. In addition it was a difficult material to handle at the mill with significantly increased unloading time. In practice for the material to be of interest to the layer sector it would need to be provided in a pelleted format for milling and transport efficiency reasons.

Key data are presented below

	Trial	**Control**
Flock size	57800	57800
Eggs per bird housed to 72 wks	317.7	314.3
Ave egg weight g	65.6	66
Cumulative % seconds	5	5
Cumulative food consumption kg	48.3	48

3.5.2. Trial objectives and basic design

As part of the of the ENNBIO project Noble Foods organised three commercial free range farm trials in their Scottish region. On two of the farms there was a concurrent control and trial flock, Kirvennie and Mains of Woodstone. On the third farm, due to the feed bin arrangement on the farm, the control was the previous flock on the farm. A total of 57,800 birds were used for both the trial and control flocks.

The diets were formulated by Premier Nutrition and were typical commercial layer diets with the inclusion of 7.5% WDDGS and appropriate enzyme supplementation. A staged feeding approach was adopted through the laying cycle as is normal practice.

The diets were all manufactured at Noble Foods mill at Thornton in Fife.

One of the concerns that we had over the use of WDDGS was the possibility of increased levels of dirty eggs. Previous experience of using maize distillers from the spirits industry had given rise to sufficient levels of eggs with a tarry manure deposit on them such that we would not use this raw material in layer diets. This was many years ago, however, and before the advent of feed enzymes. The emphasis of the spirits industry is also quite different to that of the bio-ethanol industry and the raw material base, wheat rather than maize, is different.

The small scale trials conducted at the University of Nottingham gave rise to no such egg quality problems and this gave us the confidence to proceed to farm scale commercial trials.

3.5.3. Results

Table 30 gives a summary of the flock results. In any layer trial covering the full laying cycle there is always the question of variability arising from factors other than those under test. A true statistical analysis of these results is not possible due to the limited number of replicates. However, the results all fall within the variation we would expect in commercial laying flocks.

Table 30 Diet composition and calculated analysis (% unless otherwise stated)

Raw material	218 to 40 weeks	228 41-55 weeks	238 56 weeks on
Sodium bicarbonate	0.267	0.316	0.321
Monocalcium phosphate	0.238	0.147	0.065
Soya oil	1.778	0.902	0.277
Fat blend	-	-	0.723
Limestone	9.159	9.216	10.056
Whole maize	10.000	-	-
W-DDGS	**7.500**	**7.500**	**7.500**
Salt	0.220	0.190	0.180
Soya bean meal	12.689	7.573	5.141
Sunflower seed meal	7.500	7.500	7.500
Wheat	30.037	43.529	44.315
Wheatfeed	-	2.466	3.284
Lysine HCl	0.160	0.230	0.240
Methionine	0.119	0.098	0.065
Pigment and enzyme	0.333	0.333	0.333
Barley	20.000	20.000	20.000
TOTAL	100.000	100.000	100.000

	218	228	238
Dry matter	88.946	88.893	88.972
Crude protein	17.498	16.008	14.999
Oil A	3.680	2.672	2.771
Fibre	4.452	4.565	4.562
Ash	12.611	12.478	13.142
LYS	0.848	0.778	0.719
Available LYS	0.736	0.675	0.621
MET	0.420	0.380	0.340
MET + CYS	0.739	0.686	0.633
TRP	0.214	0.197	0.183
THR	0.621	0.542	0.501
ME (MJ/kg)	11.499	11.280	11.201
Ca	3.800	3.800	4.100
Tot P	0.470	0.452	0.427
Av P	0.340	0.320	0.300
ARG	1.070	0.940	0.864

Na	0.182	0.181	0.178
NaCl	0.415	0.414	0.406
K	0.706	0.652	0.614
Cl	0.253	0.252	0.247
Linoleic acid	1.880	1.382	1.200
Cu (ppm)	17.105	16.773	16.538
Total added fats	1.778	0.902	1.000
Vit A (x 1000 IU/kg)	6.001	6.001	6.001
Vit D3 (x 1000 IU/kg)	3.000	3.000	3.000
Vit E (mg/kg)	4.998	4.998	4.998
Dig P	0.328	0.310	0.290
Dig LYS	0.735	0.675	0.621
Dig MET	0.370	0.333	0.298
Dig MET + CYS	0.635	0.587	0.540
Dig THR	0.510	0.440	0.402
Dig TRP	0.182	0.166	0.153
Dig ARG	0.955	0.832	0.762
Dig ILEU	0.598	0.522	0.480

Table 31. Performance and egg quality

Farm	Kirvennie		Mains of Woodstone		Hillocks of Gourdie		Summary	
	H2 Trial	H1 Control	H1 Trial	H2 Control	Flock 2 Trial	Flock 1 Control	Trial	Control
Flock size	16000	16000	16000	16000	25800	25800	57800	57800
Breed	ISA Warren	ISA Warren	Lohmann Brown	Lohmann Brown	Lohmann Brown	Lohmann Brown		
Age and date at housing	16 Wks	16 wks	16 Wks	16 Wks	16 Wks	16 wks		
	22/03/2013	22/03/2013	14/06/2013	14/06/2013	26/01/2013	04/11/2011		
Age and date of depletion	74 wks	74 wks	70 wks	70 wks	72 wks	74 wks		
	28/04/2014	27/04/2014	25/06/2014	12/06/2014	17/02/2014	16/02/2012		
Eggs per bird housed to 72 wks	323.7	305.5	323.4	322.8	306.0	314.7	317.7	314.3
Ave egg weight g	64.3	64.3	65.7	66.7	66.9	66.9	65.6	66.0
Cumulative % seconds	5.4	5.4	3.9	3.7	5.7	5.9	5.0	5.0
Cum Food consumption kg	45.8	46.0	50.2	46.9	49.0	51.0	48.3	48.0

Notes

Mains of Woodstone data are 70 weeks, all the rest are to 72 weeks

4. Pig nutritional and performance studies

4.1. Nottingham Pig 1

Title: The determination of ileal amino acid digestibility of Wheat Distillers Dark Grains with Solubles (W-DDGS) in grower pigs.

4.1.1. Summary

a. The data for standardised ileal digestibility of W-DDGS are not considered sufficiently robust to allow formulation for the subsequent growth trial. Possible reasons for this are based on excessive shedding of mucosal cells giving rise to very large endogenous losses thus resulting in very low or even negative coefficients of digestibility. Such losses could be linked to the effects of
    - Semi-purified diets based on starch and glucose
    - Electrical stunning during slaughter

Diet SID amino acids

	SID W-DDGS	
	Diet 3	Diet 4
LYS	0.241	0.038
MET	0.542	0.444
THR	0.510	0.373
TRY	0.475	0.369
ILEU	0.485	0.388
PHE	0.657	0.579
HIS	0.534	0.447
LEU	0.587	0.496
VAL	0.541	0.429
ARG	0.610	0.513
CYS	0.530	0.427

b. Coefficients of Apparent Total Tract Digestibilities of Gross Energy, Phosphorus and Neutral Detergent Fibre were determined following collection of faeces prior to slaughter and ranged from 0.51-0.64, 0.21-0.32 and 0.70-0.77 respectively.
c. Standardised ileal digestibility of W-DDGS was determined subsequently at Illinois, USA, using ileal cannulated pigs (see Pig Illinois Pig 1).

4.1.2. Trial objectives and basic design

Four diets with incremental increases in W-DDGS (20, 40, and 60%) with a constant level of wheat (20%); this incremental approach has been used in the past (and Nottingham work using a similar protocol has been published).
Concern has been raised over very high levels of starch and possible effects on gut mucosal cells in semi-synthetic diets.

Each diet was fed to four pigs; at the end of the trial, animals were slaughtered and ileal digesta removed; prior to this, faecal samples had been obtained.

Data for ileal amino acid digestibility were sent to EVONIK for calculation of Standardised IIeal Digestibility values that were used in the subsequent growth trial.
Analysis of faecal samples was undertaken by Nottingham (N, P) and Sciantec (GE)

Test diets

Presented in Table 32

4.1.3. Results / Conclusions

a. Amino acids

Coefficients of SID amino acids are presented in Table 33. Most for diet 1 (wheat, high starch / glucose) were negative whereas those for diet 2 were very low and sometimes negative. Data for diets 3 and 4 appeared more realistic but were still rather low.

The original intention to calculate SID for W-DDGS by regression accordingly could not be pursued.
Instead, SID for W-DDGS were calculated by difference with assumed values for wheat with data presented in Table 34.

The data were the subject of considerable discussion between members of the non-ruminant group. The general conclusion was that the amino acid digestibility data are not sufficiently robust to allow formulation of diets for the subsequent growth trial and that the facilities of Dr Hans Stein at Illinois, USA, should be used with a protocol involving ileal-cannulated pigs and semi-synthetic diets.

Coefficients of Apparent Total Tract Digestibilities (CTTAD) of other components, calculated by difference, are presented in Table 35. The general observations were:

b. GE

CTTAD for the basal diet was 0.901; for W-DDGS data varied from 0.51 – 0.64.

c. P

CTTAD for the basal diet was 0.637; for W-DDGS data varied from 0.21 to 0.32.

d. NDF

CTTAD for the basal diet was 0.632; for W-DDGS data varied from 0.70 to 0.77

Table 32 Diet composition (g.kg) and assumed analyses (%, except DE, NE MJ/kg)

	0%	**20%**	**40%**	**60%**
Wheat, ground	250			
Soya oil	50			
Limestone	6.5			
Salt	4.5			
DCP	15.5			
W-DDGS	0	200	400	600
Wheat starch	333	233	133	33
Glucose	333	233	133	33
TiO2	5			
Premix	2.5			
DM	91.62	91.54	91.46	91.38
Oil B	5.50	6.95	8.40	9.85
CP	2.75	9.27	15.79	22.31
CF	0.50	2.10	3.70	5.30
NDF	2.12	9.91	17.69	25.47
Ash	2.97	3.89	4.81	5.73
Ca	0.70	0.72	0.75	0.77
P	0.36	0.49	0.62	0.75
Dig P	0.24	0.33	0.42	0.51
Na	0.18	0.28	0.38	0.49
DE	15.86	15.74	15.62	15.50
NE	11.26	11.18	11.09	11.01

Table 33 Coefficient of Standardised Ileal Digestibility of Amino Acids

	Diet			
	1	2	3	4
MET	-0.317	0.137	0.585	0.489
LYS	-1.38	-0.35	0.33	0.13
THR	-0.748	-0.224	0.481	0.369
TRY	-0.509	-0.135	0.502	0.401
ILEU	-0.343	0.072	0.577	0.478
LEU	-0.146	0.138	0.62	0.531
VAL	-0.355	0.006	0.551	0.45
HIS	-0.183	0.093	0.582	0.493
PHE	0.112	0.272	0.678	0.604

Table 34 Coefficient of Standardised Ileal Digestibility of Amino Acids in W-DDGS determined by difference with assumed values for wheat, data presented in Figure **9**

	SID W-DDGS	
	Diet 3	Diet 4
LYS	0.241	0.038
MET	0.542	0.444
THR	0.510	0.373
TRY	0.475	0.369
ILEU	0.485	0.388
PHE	0.657	0.579
HIS	0.534	0.447
LEU	0.587	0.496
VAL	0.541	0.429
ARG	0.610	0.513
CYS	0.530	0.427

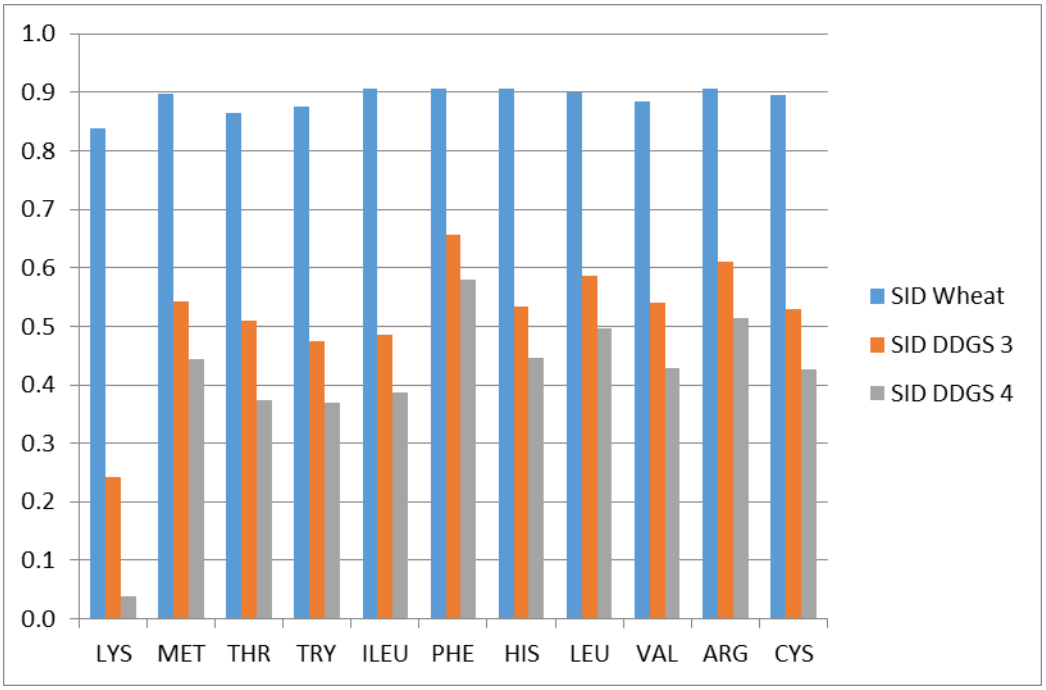


Figure 9

Table 35 Coefficient of Total Tract Apparent Digestibility of other components

a. Gross Energy

Diet	<.001
Lin	<.001
Quad	0.096

Diet			
1	2	3	4
0.901	0.835	0.746	0.718

s.e.d.	0.0149
cv%	2.6

b. Phosphorus

Diet	<.001
Lin	<.001
Quad	0.975

Diet			
1	2	3	4
0.637	0.497	0.437	0.298

s.e.d.	0.0491
cv%	14.8

c. Neutral detergent Fibre

Diet	0.002
Lin	0.002
Quad	0.005

Diet			
1	2	3	4
0.632	0.413	0.43	0.439

s.e.d.	0.0466
cv%	13.8

4.2. Illinois Pig 1

Title: Amino acid digestibility in 5 sources of DDGS from Europe fed to growing pigs.

4.2.1. Summary

a. An experiment was conducted to determine the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of amino acids (AA) by growing pigs fitted with T-cannulae in 5 different sources of DDGS from Europe. The 5 sources of DDGS include New wheat-maize DDGS, Old wheat DDGS (2012 harvest), New crop UK DDGS (2013 harvest), Hungary maize DDGS, and Germany DDGS.

b. Hungary maize DDGS had the greatest (P < 0.05) AID and SID for most AA, but Germany DDGS had the lowest (P < 0.05) AID and SID for most AA among all 5 ingredients. New

wheat-maize DDGS, Old wheat DDGS, New crop DDGS, and Germany DDGS had relatively low Lys:CP and AID and SID of Lys compared with Hungary DDGS, which indicates these ingredients may have been heat damaged.

Summary data for key amino acids presented below; more details in results

	New wheat-maize DDGS	Old Wheat DDGS	New Wheat DDGS	Hungary maize DDGS	Germany DDGS
CP,	64.5	61.37	62.99	68.64	59.79

Lys	31.89	32.11	27.13	58.85	32.06
Met	72.31	70.39	69.17	83.29	69.62
Thr	63.89	60.55	59.98	70.2	57.72
Trp	62.95	61.4	60.08	59.62	53.18

4.2.2. Trial objectives and basic design

The current trial was subsequent to Nottingham Pig 1 where the conclusion was that the protocol adopted was not sufficiently robust to generate meaningful ileal amino acid digestibility for W-DDGS.

The objective of the current experiment was to compare the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of amino acids (**AA**) by growing pigs in distillers dried grains with solubles (**DDGS**) produced from wheat or corn or wheat-corn mixtures and produced at 5 different European facilities.

Twelve growing castrates (initial BW: 23.00 ± 2.18 kg) were equipped with a T-cannula in the distal ileum and allotted to a replicated 6 × 6 Latin square design with 6 diets and six 7-day periods in each square. There were 12 replicate pigs per treatment. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens have smooth sides and fully slatted tribar floors. A feeder and a nipple drinker are installed in each of the pens.

All pigs were allowed ad libitum access to their diets throughout the experiment and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was also recorded. The initial 5 days of each period was considered an adaptation period to the diet. Ileal digesta were collected for 8 hours on days 6 and 7 using standard operating procedures. In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected.

Test diets

Presented in Table 36

4.2.3. Results / Conclusions

AID and SID data are presented in Table 37 and Table 38 respectively.

The AID and the SID of all AA in the 2 sources of wheat DDGS were less than previously been reported.

There are only limited data on the AID and SID of AA in wheat-maize DDGS but the data from the present experiment indicate the AID and SID of AA in wheat-maize DDGS are close to the values for wheat DDGS. Both sources of wheat-maize DDGS had AID and SID of AA that were less than the values reported for Canadian wheat-maize DDGS.
One of the challenges in producing DDGS with a high SID of AA is to avoid heat damage of DDGS during the drying procedure.

Because heat damage will reduce the digestibility of primarily Lys, the heat damage during processing of these sources of DDGS is probably responsible for the very low values for SID of Lys that were observed in both sources of wheat DDGS. However, heat damage will reduce the SID of not only Lys but also other AA although the reduction in SID is less for other AA than for Lys. It is therefore probable that the reason for the reduced SID of most AA obtained in wheat DDGS used in this experiment compared with previous sources of wheat DDGS is a result of the heat damage done to these ingredients.

Table 36 Composition of experimental diets (%)

Ingredient	DDGS diets	N-free diet
DDGS	50.00	-
Cornstarch	25.5	67.80
Sucrose	20.00	20.00
Soybean oil	2.00	4.00
Solka floc	-	4.00
Monocalcium phosphate	-	2.0
Limestone	1.40	0.60
Chromic oxide	0.40	0.40

Salt	0.40	0.40
Vitamin –mineral premix[2]	0.30	0.30
Magnesium oxide	-	0.10
Potassium carbonate	-	0.40
Total	100.00	100.00

Table 37 Analyzed composition of 5 dried distillers grains with solubles

Item	New wheat-corn DDGS	Old Wheat DDGS	New Wheat DDGS	Hungary Corn DDGS	Germany DDGS
DM, %	90.00	89.53	90.71	88.71	90.25
CP, %	30.67	32.35	34.60	29.01	28.74
ADF, %	21.86	24.49	24.83	13.35	17.89
NDF, %	33.66	33.68	35.24	27.13	30.42
Indispensable AA, %					
Arg	1.09	1.23	1.26	1.26	1.04
His	0.57	0.58	0.62	0.76	0.57
Ile	1.01	1.11	1.16	1.05	0.97
Leu	2.26	2.15	2.24	3.45	2.18
Lys	0.49	0.53	0.53	0.84	0.56
Met	0.43	0.46	0.46	0.60	0.42
Phe	1.36	1.45	1.56	1.43	1.26
Thr	0.89	0.96	0.98	1.07	0.90
Trp	0.28	0.32	0.34	0.23	0.27
Val	1.30	1.40	1.46	1.39	1.25
Mean	9.68	10.19	10.62	12.08	9.42
Dispensable AA, %					
Ala	1.36	1.21	1.26	2.13	1.30
Asp	1.53	1.59	1.63	1.89	1.53
Cys	0.52	0.61	0.60	0.55	0.49
Glu	7.11	8.47	9.09	5.21	6.37
Gly	1.19	1.30	1.35	1.15	1.11
Pro	2.56	2.85	3.07	2.36	2.33
Ser	1.29	1.44	1.47	1.42	1.21

Table 38 Apparent ileal digestibility (AID) of crude protein (CP), and amino acids (AA) in dried distillers grains with solubles (DDGS) by growing pigs

Item	New wheat-maize DDGS	Old Wheat DDGS	New Wheat DDGS	Hungary maize DDGS	Germany DDGS	SEM	*P*-value
CP,	53.40^{ab}	51.72^{bc}	53.44^{ab}	57.43^{a}	48.22^{c}	2.1	<0.05
Indispensable AA, %							
Arg	65.93^{b}	65.86^{b}	66.69^{b}	76.00^{a}	65.86^{b}	3.35	<0.01
His	65.60^{b}	62.86^{bc}	63.66^{b}	74.26^{a}	59.98^{c}	1.72	<0.01
Ile	64.57^{b}	63.34^{b}	62.70^{b}	69.79^{a}	57.56^{c}	1.71	<0.01
Leu	73.36^{b}	68.71^{c}	68.20^{c}	83.75^{a}	68.54^{c}	1.41	<0.01
Lys	17.31^{b}	20.75^{b}	14.65^{b}	51.08^{a}	20.76^{b}	3.87	<0.01
Met	67.55^{b}	66.59^{b}	64.97^{b}	80.31^{a}	65.17^{b}	1.57	<0.01
Phe	69.41^{b}	68.88^{b}	68.28^{bc}	74.09^{a}	65.28^{c}	1.73	<0.01
Thr	51.92^{b}	50.53^{bc}	49.63^{bc}	60.76^{a}	46.38^{c}	2.17	<0.01
Trp	53.13^{a}	53.71^{a}	52.09^{ab}	47.22^{b}	42.38^{c}	2.32	<0.01
Val	62.02^{b}	60.33^{bc}	59.02^{bc}	69.76^{a}	57.02^{c}	1.83	<0.01
Mean	63.46^{b}	61.32^{bc}	60.81^{bc}	73.23^{a}	58.72^{c}	1.78	<0.01
Dispensable AA, %							
Ala	53.37^{b}	43.41^{c}	42.37^{c}	70.13^{a}	50.29^{b}	2.64	<0.01
Asp	45.07^{b}	39.83^{bc}	38.39^{c}	61.77^{a}	41.19^{bc}	2.68	<0.01
Cys	62.16^{b}	61.94^{b}	61.53^{b}	67.75^{a}	55.16^{c}	1.9	<0.01
Glu	81.55^{a}	80.82^{ab}	81.98^{a}	79.20^{b}	76.79^{c}	1.02	<0.01
Gly	28.39	27.47	29.43	19.64	17.42	5.62	0.06
Pro	7.76^{a}	17.14^{a}	22.03^{a}	-17.52^{b}	3.32^{ab}	16.6	<0.01
Ser	66.68^{b}	64.63^{b}	66.16^{b}	73.23^{a}	60.20^{c}	1.77	<0.01
Mean	57.39	57.84	59.46	54.56	51.79	3.64	0.09
All AA	59.69^{a}	59.05^{ab}	59.93^{a}	62.95^{a}	54.52^{b}	2.67	<0.05

$^{a-c}$Within a row, means without a common superscript letter are different ($P < 0.05$).

Table 39 Standardized ileal digestibility (SID) of dry matter (DM), crude protein (CP), and amino acids (AA) in dried distillers grains with solubles (DDGS) by growing pigs

Item	New wheat-maize DDGS	Old Wheat DDGS	New Wheat DDGS	Hungary maize DDGS	Germany DDGS	SEM	*P*-value
CP, %	64.50^{ab}	61.37^{b}	62.99^{b}	68.64^{a}	59.79^{b}	2.1	<0.05
Indispensable AA, %							
Arg	74.31^{b}	72.43^{b}	73.54^{b}	80.80^{a}	74.08^{b}	3.35	<0.01
His	71.47^{b}	68.00^{bc}	68.80^{bc}	78.44^{a}	65.61^{c}	1.72	<0.01
Ile	70.45^{b}	68.03^{b}	67.64^{b}	75.18^{a}	63.37^{c}	1.71	<0.01
Leu	77.76^{b}	72.89^{c}	72.47^{c}	86.51^{a}	72.95^{c}	1.41	<0.01
Lys	31.89^{b}	32.11^{b}	27.13^{b}	58.85^{a}	32.06^{b}	3.87	<0.01
Met	72.31^{b}	70.39^{b}	69.17^{b}	83.29^{a}	69.62^{b}	1.57	<0.01
Phe	77.27^{b}	75.55^{bc}	74.83^{bc}	81.23^{a}	73.36^{c}	1.73	<0.01
Thr	63.89^{b}	60.55^{bc}	59.98^{bc}	70.20^{a}	57.72^{c}	2.17	<0.01
Trp	62.95^{a}	61.40^{a}	60.08^{a}	59.62^{a}	53.18^{b}	2.32	<0.01
Val	68.34^{b}	65.50^{bc}	64.42^{bc}	75.38^{a}	63.23^{c}	1.83	<0.01
Mean	70.64^{b}	67.39^{bc}	67.07^{bc}	78.69^{a}	65.73^{c}	1.78	<0.01
Dispensable AA, %							
Ala	62.57^{b}	52.73^{c}	51.83^{c}	75.77^{a}	59.56^{b}	2.64	<0.01
Asp	54.61^{b}	48.00^{c}	46.86^{c}	69.01^{a}	50.20^{bc}	2.68	<0.01
Cys	69.78^{b}	67.78^{b}	67.84^{b}	74.52^{a}	62.82^{c}	1.9	<0.01
Glu	84.18^{a}	82.81^{a}	83.95^{a}	82.61^{a}	79.59^{b}	1.02	<0.01
Gly	54.56	49.03	51.29	45.43	44.46	5.62	0.26
Pro	49.93	50.54	55.27	26.4	46.96	16.6	0.08
Ser	74.30^{b}	70.89^{bc}	72.49^{b}	79.81^{a}	68.01^{c}	1.77	<0.01
Mean	70.16	68.07	69.71	67.42	65.02	3.63	0.42
All AA	70.33^{a}	67.75^{ab}	68.74^{ab}	72.48^{a}	65.30^{b}	2.67	<0.05

$^{a-c}$Within a row, means without a common superscript letter are different ($P < 0.05$).

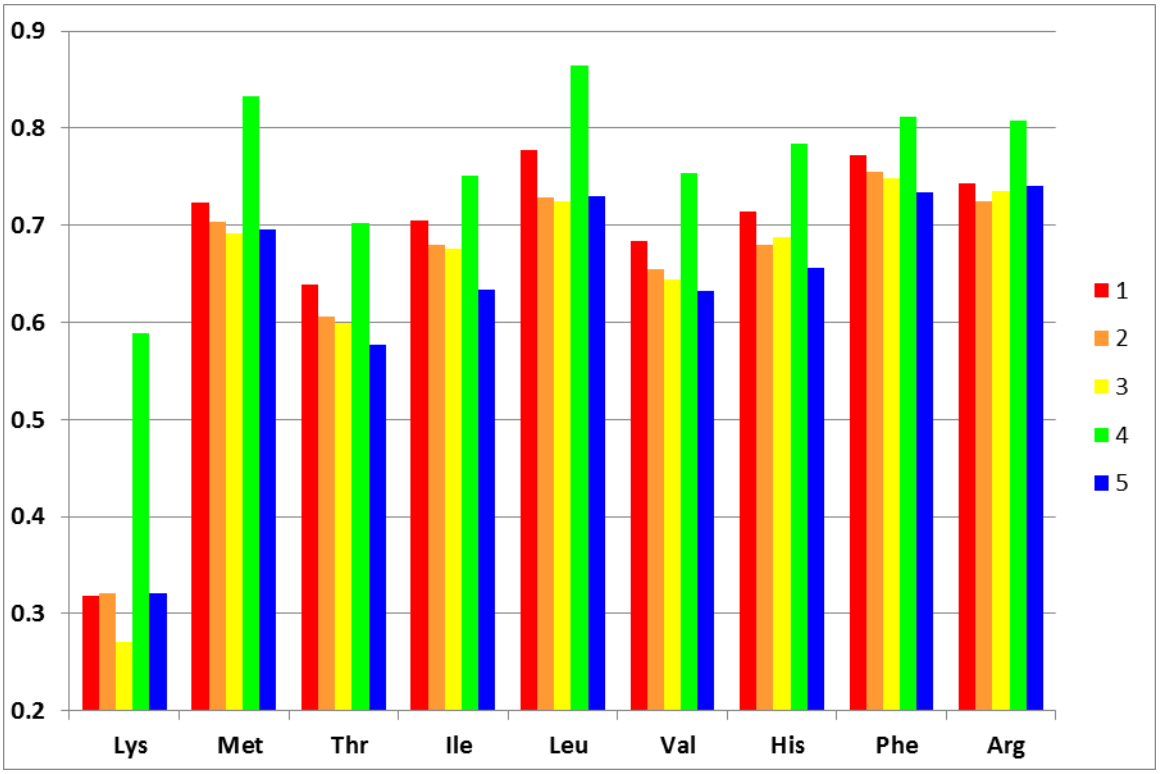


Figure 10 Coefficient of SID of indispensable amino acids (data from **Table 39**, converted from %)

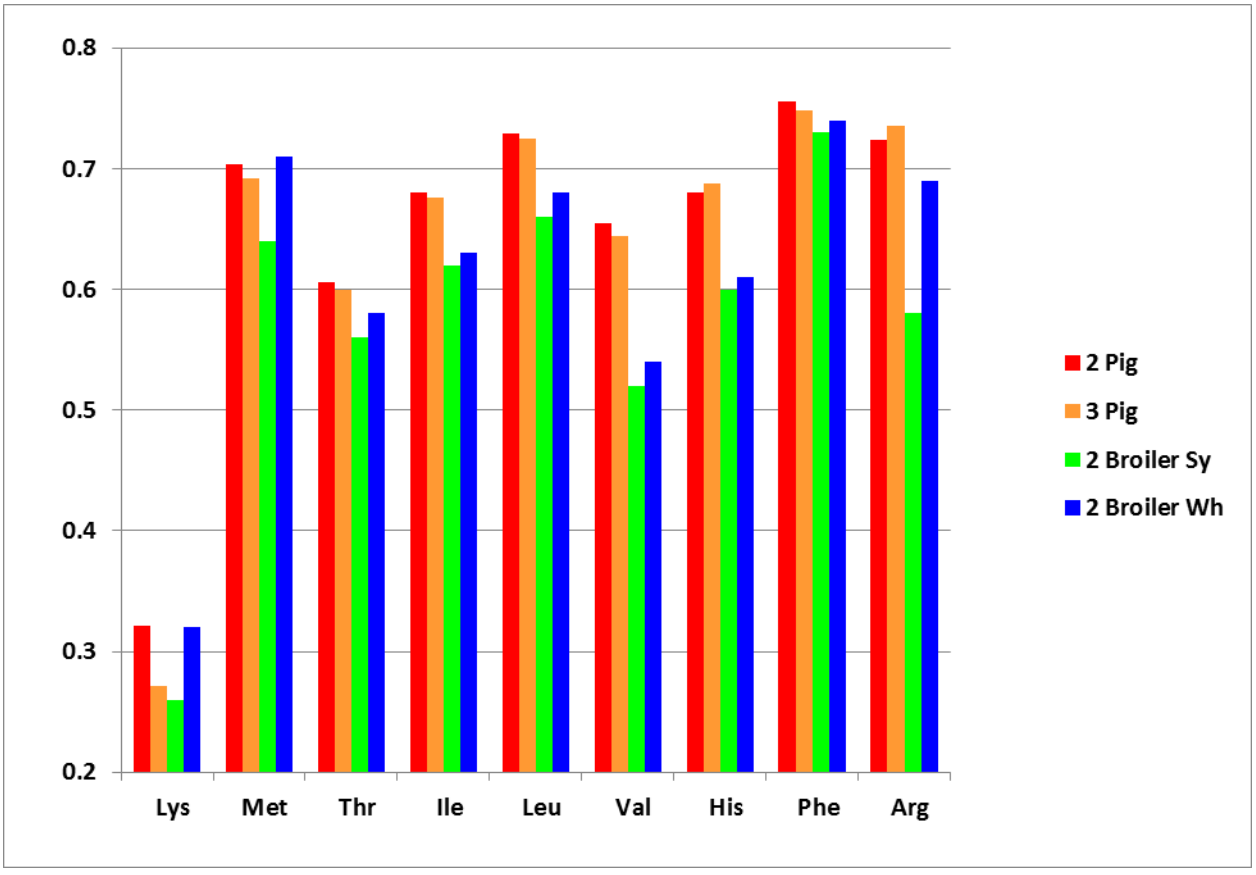


Figure 11 Coefficient of SID of indispensable amino acids in Old Wheat DDGS: Illinois (2) vs Nottingham Pig 1 (3), Nottingham Broiler 2 (Synthetic and Wheat basal respectively)

4.3. Nottingham Pig 2

Title: Determination of growth rate, feed intake and feed conversion ratio between 35-65kg (grower) and 65-105kg (finisher) live weight in entire male pigs fed four commercial diets containing different levels of W-DDGS.

4.3.1. Summary and conclusions

a. In conclusion growing / finishing pigs are able to tolerate levels of W-DDGS up to 300g/kg in pelleted balanced diets in terms of performance and carcass quality (including pH changes post mortem and shoulder fat skatole levels) without a significant reduction in performance.

b. On a 'practical' level, there was a strong trend for a quadratic effect (P=0.063 and 0.058 for grower and finisher; overall no effect, P=0.202) of rate of inclusion of W-DDGS on DLWG, suggesting that a maximum of 200g/kg would be suitable. There were no similar trends for FI and FCR.

Key performance data are presented below

	DLWG kg			Total FI kg			FCR		
	Phase			Phase			Phase		
W-DDGS (g/kg)	Grower	Finisher	Overall	Grower	Finisher	Overall	Grower	Finisher	Overall
0	1.03	1.11	1.09	58	109	188	1.94	3.10	2.51
100	1.10	1.19	1.14	56	110	190	1.86	3.15	2.53
200	1.08	1.18	1.13	56	105	182	1.86	3.01	2.42
300	1.04	1.08	1.04	60	108	191	1.99	3.07	2.54

Key measurement data

W-DDGS (g/kg)	KO%	P2 mm	Length mm	pH			Skatole µg/g	Indole µg/g
				45 m PM	24 h PM	Change		
0	74.2	8	842	6.2	5.8	0.4	0.037	0.021
100	74.2	7	845	6.2	5.8	0.5	0.032	0.019
200	74.9	8	826	6.2	5.9	0.3	0.041	0.047
300	74.1	6	845	6.3	5.9	0.3	0.032	0.033

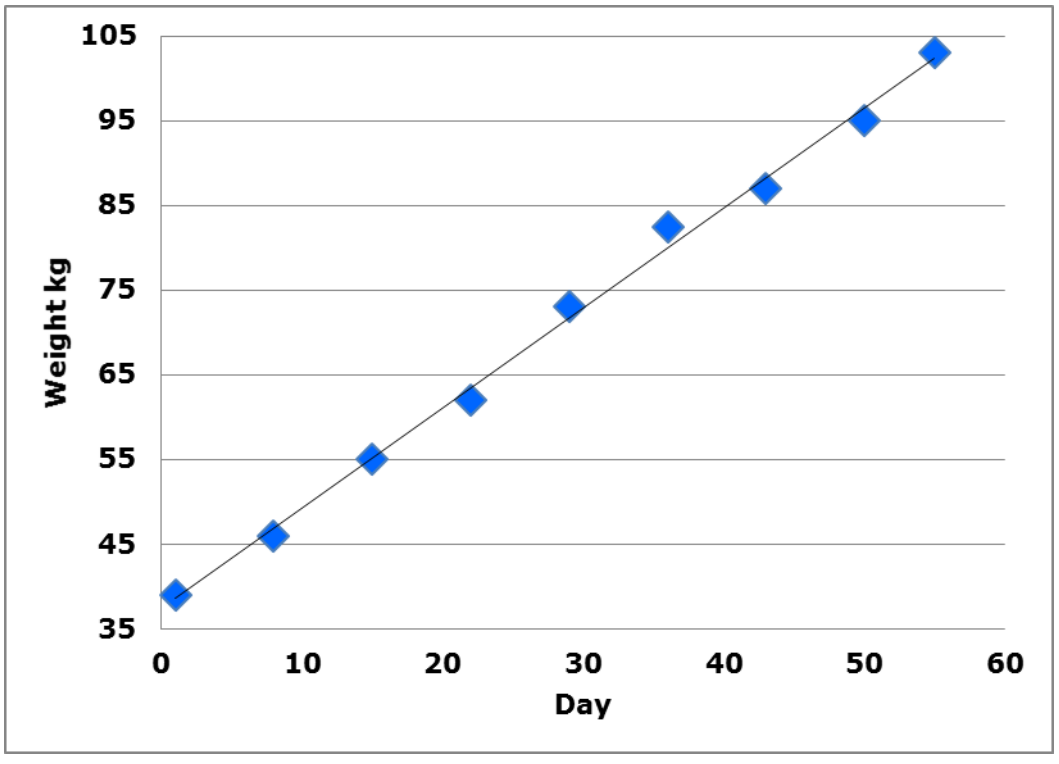
4.3.2. Trial Objectives and Basic Design

The trial was a 'production' programme designed to examine performance of growing / finishing pigs fed diets containing graded levels of W-DDGS in isocaloric and isonitrogenous diets balanced for standardised ileal digestible amino acids.

The study was conducted with growing finishing entire male pigs housed individually in the Pig Research Unit at the University of Nottingham. Animals were slaughtered in the on-site abattoir for assessments of carcass quality.

Animals of approximately 35 kg were introduced and offered the experimental grower diets (nine pigs per treatment). Feed intake and liveweight were measured on a weekly basis. Once an animal had approached 65kg on the weekly weighing, it was transferred to the same finisher diet (i.e. an animal on a grower diet with a specific rate of inclusion of W-DDGS was transferred to the same rate for the finisher phase).

Daily liveweight gain was estimated by the linear regression of time against weight, as illustrated in Figure 12.



- Linear response gives DLWG (slope) and intercept (weight at day zero): y = 1.18x + 37.3
- This allows the exact time at weights 35, 65 and 105 to be estimated
  Feed intake is adjusted accordingly.
  FCR is total feed intake / LW range (70)
  For grower and finisher phases, the same procedure is adopted

Figure 12 Calculation of daily liveweight gain (example is 35-105kg liveweight)

Incremental increases in a factor (in this case W-DDGS) need to be analysed with a POLYANOVA model partitioning variance into linear and non-linear (in this case quadratic) contrasts.

Thus there will be overall P values together with those associated with a linear and quadratic response.

Test Diets

Four experimental grower and finisher diets (0, 100, 200, 300g W-DDGS/kg were formulated and manufactured by ABAgri. Details are in Appendix 1.

4.3.3. Results

Performance:

Table 40 (a) Daily liveweight gain (DLWG, kg) together with (b) total feed intake (TFI, kg) and (c) Feed Conversion Ratio (FCR) over the relevant phase (kg) and (d) Daily Feed Intake

(a)

	Phase		
W-DDGS (g/kg)	Grower	Finisher	Overall
0	1.03	1.11	1.09
100	1.10	1.19	1.14
200	1.08	1.18	1.13
300	1.04	1.08	1.04
	ANOVA		
S.E.D	0.04	0.097	0.058
P	0.259	0.618	0.286
P (LIN)	0.939	0.768	0.357
P (QUAD)	0.063	0.202	0.090

(b)

	Phase		
W-DDGS (g/kg)	Grower	Finisher	Overall
0	58	109	188
100	56	110	190
200	56	105	182
300	60	108	191
	ANOVA		
S.E.D	4.6	5.3	5.9
P	0.794	0.821	0.438
P (LIN)	0.776	0.621	0.989
P (QUAD)	0.341	0.936	0.374

(c)

	Phase		
W-DDGS (g/kg)	Grower	Finisher	Overall
0	1.94	3.10	2.51
100	1.86	3.15	2.53
200	1.86	3.01	2.42
300	1.99	3.07	2.54
	ANOVA		
S.E.D	0.154	0.152	0.078
P	0.793	0.821	0.439
P (LIN)	0.775	0.652	0.991
P (QUAD)	0.34	0.936	0.373

(d). Daily feed intake requested); this is calculated as the number of days taken to grow the specific liveweight range (LW range / DLWG) divided by the total feed consumed for that range.

	Phase		
W-DDGS (g/kg)	Grower	Finisher	Overall
0	2.01	3.39	2.72
100	2.03	3.72	2.88
200	1.99	3.50	2.72
300	2.04	3.31	2.63
	ANOVA		
S.E.D	0.316	0.532	0.282
P	0.987	0.395	0.329
P (LIN)	0.888	0.555	0.310
P (QUAD)	0.913	0.152	0.211

Carcass quality

W-DDGS (g/kg)	KO%	P2 mm	Length mm	pH			Skatole µg/g	Indole µg/g
				45 m PM	24 h PM	Change		
0	74.2	8	842	6.2	5.8	0.4	0.037	0.021
100	74.2	7	845	6.2	5.8	0.5	0.032	0.019
200	74.9	8	826	6.2	5.9	0.3	0.041	0.047
300	74.1	6	845	6.3	5.9	0.3	0.032	0.033
	ANOVA							
S.E.D	0.69	1.3	9.9	0.08	0.09	0.13	0.0172	0.0075
P	0.619	0.540	0.173	0.761	0.404	0.716	0.940	0.005
P (LIN)	0.820	0.516	0.705	0.368	0.186	0.709	0.897	0.015
P (QUAD)	0.460	0.528	0.246	0.661	0.673	0.565	0.885	0.249

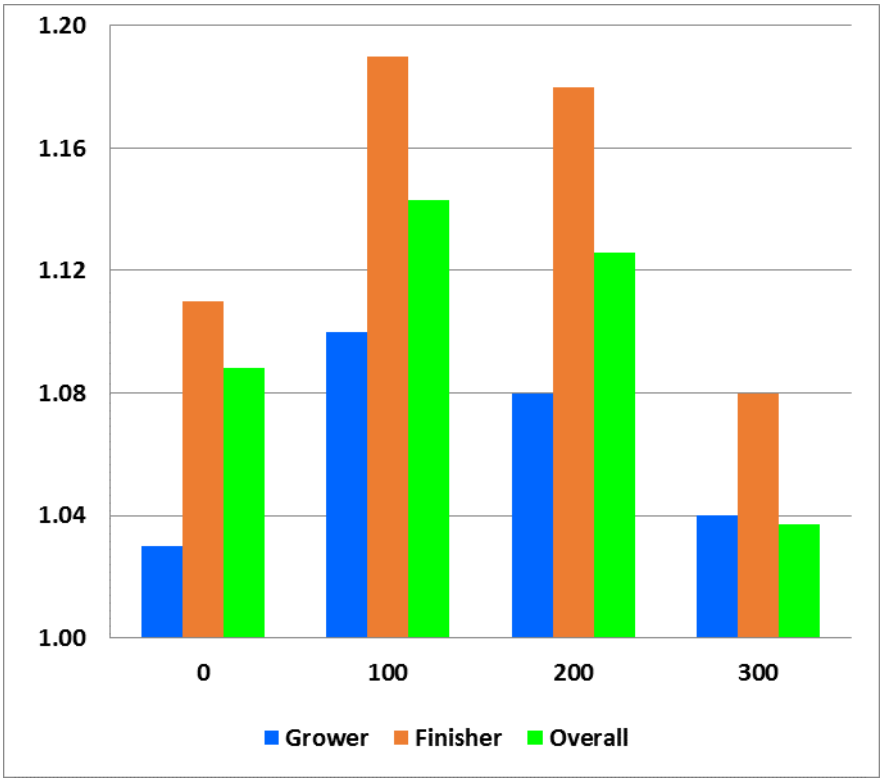


Figure 13 Daily liveweight gain (kg) y axis, x axis data are rate of inclusion of W-DDGS (g/ WDDGS/kg diet)

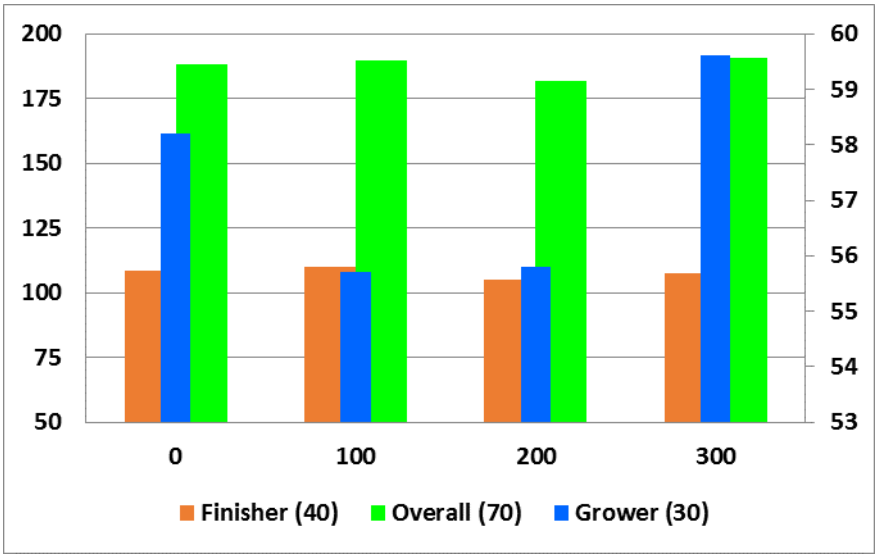


Figure 14 Feed intake (kg) left y axis, over each time period (data in parentheses are LWG, kg); grower right axis

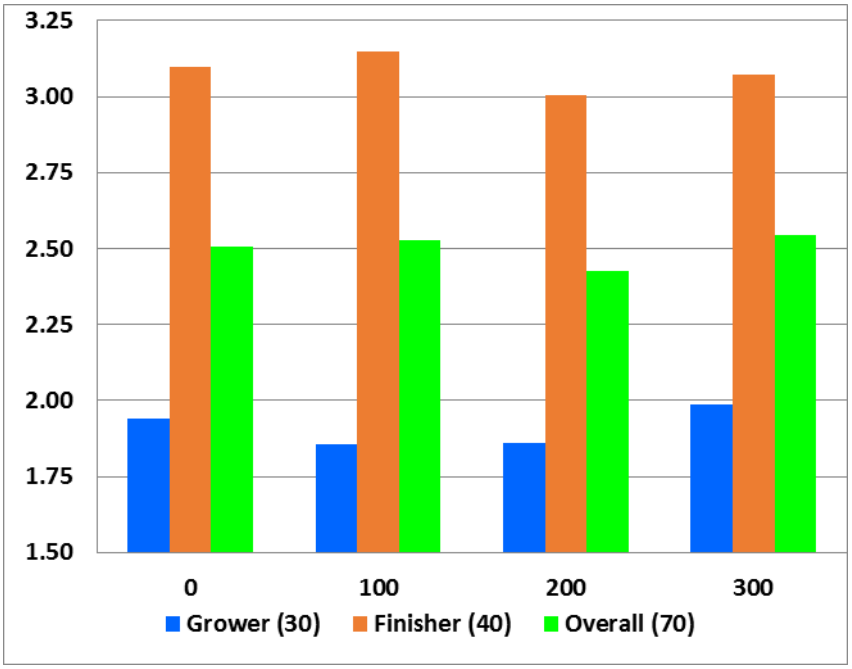


Figure 15 Feed conversion ratio y axis over each time period (data in parentheses are LWG, kg, with LW range: grower 35-65kg and Finisher 65-105kg)

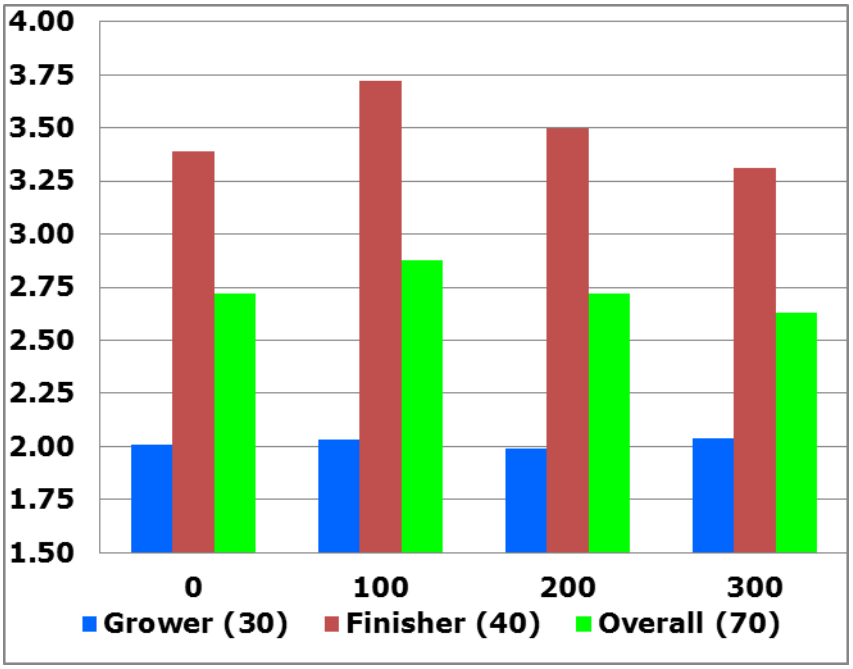


Figure 16 Daily feed intake (kg) y axis with LW range: grower 35-65kg and Finisher 65-105kg)

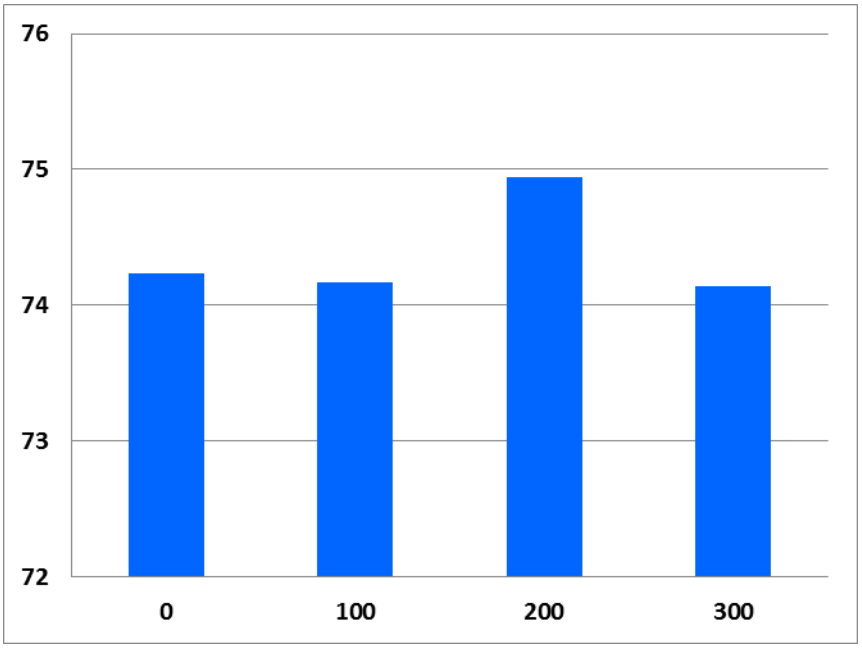


Figure 17 KO% (Fed LW / 24hr post mortem LW at 4 C 24)

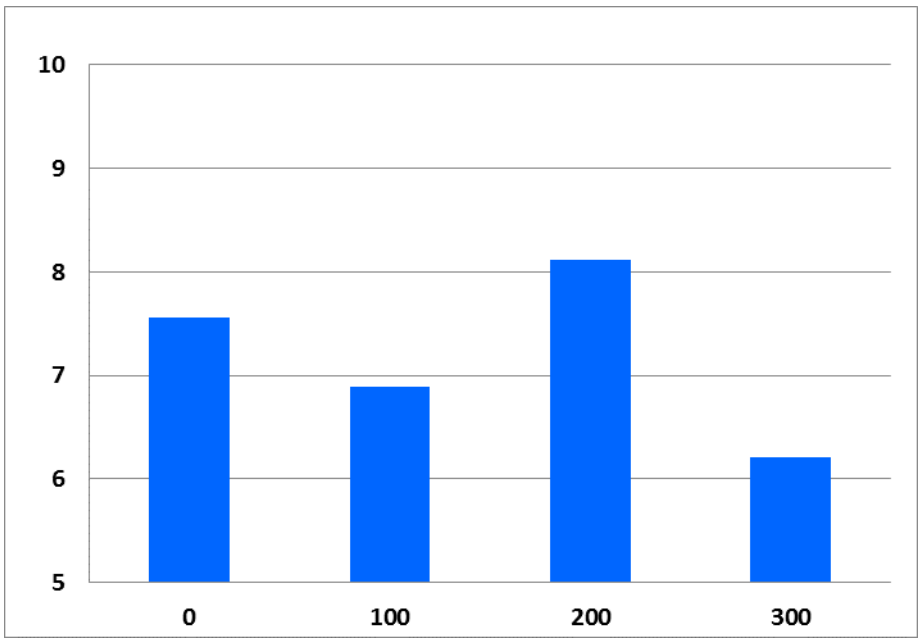
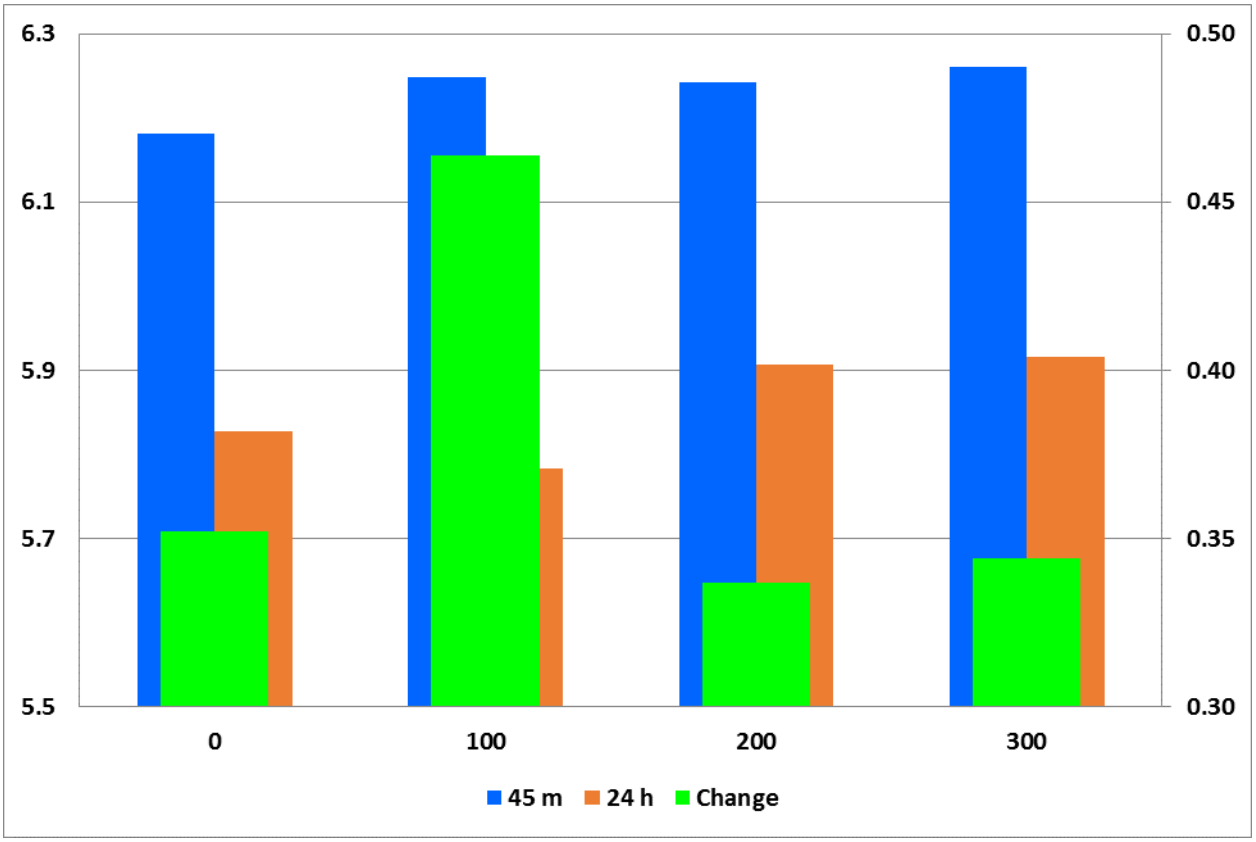
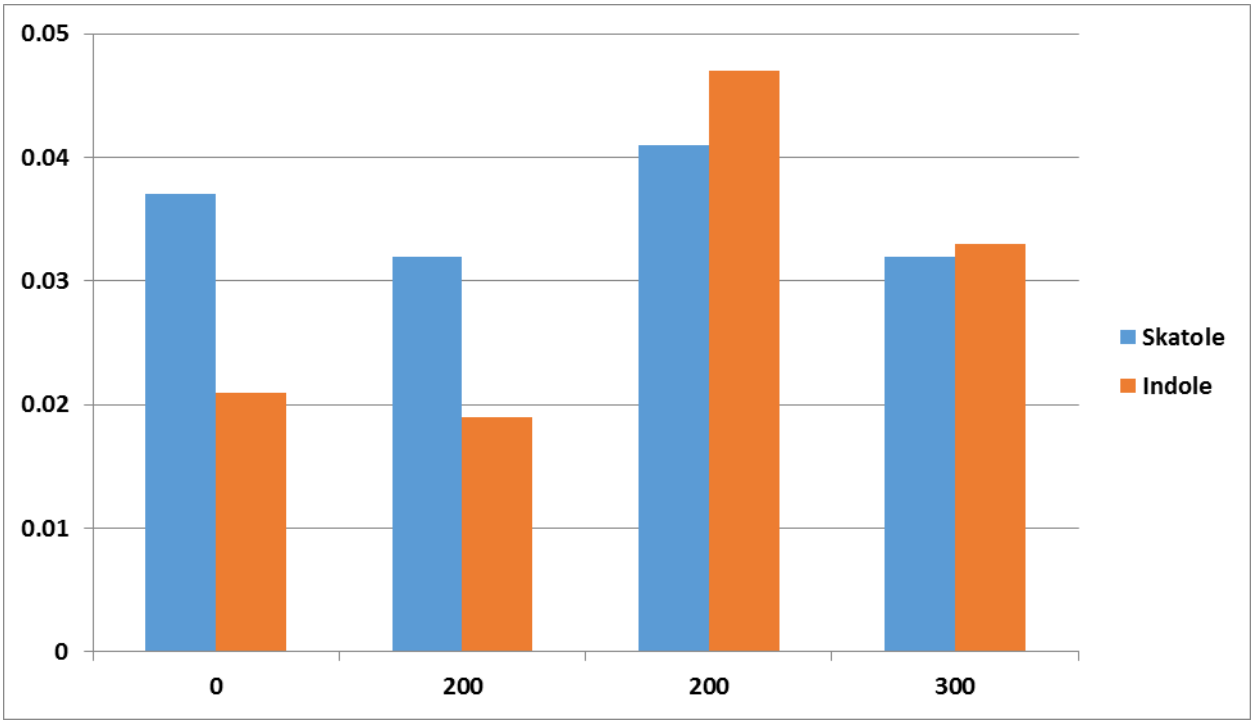


Figure 18 P2 (mm); with cold carcass weight as covariate



Note: *Lowering of pH is due to glycolysis*
- PSE increased risk < 6 pH 45m, <5.6 24h
- DFD increased risk > 6.5
-Although data are presented, change of pH is less important than at 24 hours post mortem

Figure 19 pH; 45 minutes and 25 hours post mortem and (right axis) change between these two time points



The threshold for carcass taint is 0.2 µg/g, so maximum level is approximately 20% of taint threshold

Figure 20 Skatole and indole data (µg/g)

4.4. HAU Tulip Ltd Pig Trial 1

Title: Effect of increasing dietary inclusions of Wheat Dried Distillers Grains on the performance of finishing pigs under commercial practice conditions.

4.4.1. Summary

The inclusion of Wheat Dried Distillers Grains (W-DDGS) at any of the levels in the pelleted diets did not have any negative effects for on-farm performance, slaughter characteristics or meat quality.
The only significant relationship within the dose response range and structure was FCR in the first two periods with a linear response. All other significant treatment differences could not be explained as part of a dose response.

Treatment significances showed early growth differences that affected the overall results. This seemed to be related to early gains in feed efficiency, possibly indicating better gut health and function.

No significant treatment effects on P2 corroborate the non-significant performance results in the later periods. It is interesting to note that the zero % W-DDGS inclusion control showed poorer performance than the other treatment levels both on farm and in the abattoir. The highest inclusion at 30% W-DDGS showed best performance in a number of areas including, daily liveweight gain, FCR and slaughter weight. It can therefore be concluded that feeding pigs during the growing and finishing stages with up to 30% W-DDGS included in the diets has no significant negative effect on performance. It may also be argued that a future trial may wish to look at a higher inclusion level to determine the optimum inclusion. This trial does not take into account the cost of production and at what inclusion level the W-DDGS would be most economically beneficial.

4.4.2. Trial objectives and basic design

The objective of the trial is to evaluate W-DDGS under commercial conditions as a supplementary trial to Nottingham Pig 2.

Animals

Genotype

Harper Adams has two Dam lines mated to terminal sire lines. Both dam line genotypes are representative of the UK national herd and were balanced across all treatments, JSR Genepacker 90 (LWxLR) and PIC Camborough ((LWxLR) x White Duroc). Sire breed type was representative of UK terminal sires and all breed types were balanced across treatments.

Selection

900 pigs were weighed, tagged and selected at approximately 25kg at 9 weeks of age, following weaning at 26 days of age. A total of 36 pens of finishing pens were established over three discrete batches at 3 week intervals (9 reps of 4 treatments, 3 reps per batch). Numbers of pigs in each batch was balanced across reps and treatments. Within each batch, pen weight and pig numbers were equalised over all pens. Mixed sexed groups were established with the same total number of males and females balanced across treatments. From the available batch of up to 350 pigs a selection of 300 pigs was made excluding the smallest and largest thus minimising within pen variation.

4.4.3. Pig House and Pen Allocation

Fully slatted finishing accommodation was used in rooms/batches of 12 pens (treatments were balanced within rooms). Up to 25 pigs were placed in each pen. Pens were balanced for weight, sex,

and breed type. A total of 36 pens were used in a 4 x 9 design. Once the initial pens were established at 25kg there was no further changes to pen dynamics until selection for slaughter, except in cases where pigs were removed on welfare grounds.

Treatments and Feed Regime

At the initial allocation all pens received the same commercial grower diet containing no W-DDGS. At 12 weeks of age weighing approximately 40-45kg all pigs were weighed and a final allocation of pens to one of four finishing regimes was made balancing pen weight to treatment. At this point residual grower diet in the feeders was removed and weighed and the trial diets introduced.

At 12 weeks of age the pigs were allocated to one of 4 treatment regimes. Each treatment regime consisted of two diets Finisher 1 and Finisher 2 resulting in a total of 8 trial diets. The pigs were weighed at week 15, at approximately 60 kg and the diet was changed from Finisher 1 to Finisher 2. All diets were formulated and manufactured by ABAgri, details are in Appendix 1 of the Nottingham 2 Pig Trial as the same diets were used for both studies.

- A = 0 DDG
- B = 10% DDG
- C = 20% DDG
- D = 30% DDG

4.4.4. Measurements and Records

Performance data was collected in three production periods Grower 25-40kg (12 weeks), Finisher 1 40-60kg (12-15 weeks), Finisher 2 60-90kg (15-20 weeks) and Finisher 3 90kg – Slaughter (20-21 weeks).

Weight

Pigs were weighed individually at week 9 (24kg), Grower 40kg (week 12), Finisher 1 60kg (week 15), Finisher 2 93kg (week 20) and at slaughter (98kg) (week 20). Pigs were sent to slaughter in 1 group per batch.

Feed Intake and Efficiency

Throughout the trial all feed added to the hoppers was weighed and recorded. At each weighing event, feed remaining in the hopper was weighed and used to calculate the weekly feed intake of the pigs; FCR was calculated.

Environmental Monitoring

The environment was precisely controlled on farm as per commercial practice daily temperature records were taken and action taken should any deviation occur. The temperature controls were set at 22C at entry and dropped to 20C by Slaughter.

Pig Slaughter performance

Pigs were individually slap marked and slaughtered at Tulip Ltd, Ashton-Under-Lyme.

- Hot weight was measured on the scale at the abattoir.
- P2 (backfat) measurements were taken at the abattoir.
- Leg pH measurement was measured at 45mins post slaughter and again at 24 hours post slaughter using a pH meter.
- Kill out % was calculated for each pig using the live weight measured on arrival at the abattoir and the corresponding hot weight measured on the scale.
- Cold weight was a 2% deduction of hot weight, as is industry standard.
- Lean meat % was calculated using the back fat P2 measurement and cold weight (hot weight – 2%), as shown in the following equation:

$$66.5 – (0.95*probe) + (0.068*cold\ weight)$$

- Drip loss:
  Loin chops were taken from each of the groups of pigs on trial and weighed. The chops were individually placed in a drip tray containing a drip pad and refrigerated for 24 hours. The chops were then re-weighed and drip loss calculated.

4.4.5. Statistical Analysis and Result Records

Performance data was analysed using Genstat. Analysis of variance (ANOVA) and the least significant difference (lsd) test was used in order to determine differences between inclusion rates. Furthermore an analysis of dose response was analysed using Genstat ANOVA polynomial contrast

model. Where there are significant deviations occurring then any linear and quadratic relationships should be treated with care. Differences between treatments was considered significant at $P < 0.05$. Tendencies towards significance ($0.05 \leq P < 0.1$) were reported. Results were analysed for treatment by sex with batches used as a blocking factor.

Growth Rate

During period 1 of the trial, the average growth rate was 850.5g/pig/day, at this stage there was a significant difference (P 0.002) between the treatments, with 10% inclusion treatment had a significantly higher growth rate than the control (0%) inclusion of 884g/day and 806g/day respectively (see Table 4). This may indicate improved gut function in period 1 of the trial. This was not seen throughout the rest of the trial. However, overall daily gain was still significant (P 0.039), control performed poorest at 854g/pig/day and 30% inclusion performed best at 900g/pig/day.

Feed Intake

Throughout the trial, feed intake was not significantly different at any stage. However there was a trend toward the 20% inclusion treatment having a lower feed intake than all other treatments (P = 0.082).

FCR

FCR was significant in periods 1 and 2 of the trial but not in Period 3. Overall it was significant (P = 0.038) with the 30% inclusion performing best at 2.50 and 0% inclusion performing poorest at 2.62.

Average Weights

On farm performance of average weights showed no significant difference between treatments during Period 1. Throughout Periods 2, 3 and at the final on farm weighing at slaughter there was a significant difference between treatments for each period.

Slaughter Characteristics

Carcass weights recorded at the abattoir show a significant difference in slaughter weight (P = 0.021) with the lightest pigs from 0% inclusion at 99.7kg and heaviest pigs from 30% inclusion at 102.2kg. However, the kill out percentage was not significant. Drip loss and pH were also showed no significant differences between treatments. This demonstrates that including W-DDGS up to 30% inclusion in the finisher diets has no effects on the meat quality.

Appendix A, describes the pig flow and although mortality levels were as expected the number of pigs that were removed from the trial under the removals and light weight protocol were higher than

expected. There were, however, no treatment differences in morbidity and the final batch performed as expected. The majority of removals recovered. Overall performance compares well with national performance figures (40-100 kg) of the top 1/3 indoor unit; DLWG 900g/d, FCR 2.6 and mortality 2.7 (BPEX Pig Year Book 2013-14).

Feed analysis is presented in Nottingham Pig 2 as both trials used the same diets. Growth by pen is presented in Table 41. The data includes all pigs up to the point that they left the pen, plus the small pigs (7 across all treatments) not included in the slaughter data. The data prior to the inclusion of the W-DDGS diets (period from 25-40kg) when the pigs were fed commercial grower is detailed in Appendix 5. The data regarding sex effects is also included in the appendix. As the pigs were not in split sexed pens we cannot determine the daily intake and FCR of the sexes, however growth rate and slaughter information has still been obtained and may be useful from a commercial perspective.
Means with different superscripts are significant to $P<0.050$.

Table 41. Effect of DDGS on the performance of finishing pigs (pen data analysis)

Treatment	Trial Start Weight (Kgs)	Live weight Gain (g/pig/day)	Feed Intake (g/pig/day)	FCR	Slaughter Weight (Kgs)
0	39.64	854[a]	2250	2.62[a]	98.86[ab]
10	39.68	890[ab]	2273	2.56[ab]	100.98[bc]
20	39.6	850[a]	2175	2.57[ab]	98.30[a]
30	39.67	900[b]	2236	2.50[b]	102.40[c]
P value	0.994[NS]	0.039*	0.082[TREND]	0.038*	0.013*
Linear P Value	0.813[NS]	0.140[NS]	0.257[NS]	0.008**	0.083[TREND]
Quad P value	0.947[NS]	0.614[NS]	0.485[NS]	0.870[NS]	0.352[NS]
sed	0.349	20.0	37.8	0.040	1.212
Dev P Value	0.884[NS]	0.014*	0.025*	0.243[NS]	0.006**

Table 42. Effect of DDGS on average weights (kg) of growing and finishing pigs

Treatment	Period 1	Period 2	Period 3	Slaughter
Age (weeks)	**12**	**15**	**19**	**21**
0	39.64	56.97[a]	81.24[a]	98.86[ab]
10	39.68	58.60[b]	83.66[b]	100.98[bc]
20	39.6	57.60[a]	81.30[a]	98.30[a]
30	39.67	58.27[b]	83.78[b]	102.04[c]
P Value	0.99[NS]	0.015*	0.004**	0.013*
Linear P Value	0.813[NS]	0.088[NS]	0.024*	0.083[TREND]
Quad P Value	0.947[NS]	0.199[NS]	0.671[NS]	0.352[NS]
sed	0.349	0.490	0.968	1.212
Dev P Value	0.884[NS]	0.010**	0.003**	0.006**

Table 43. Effect of DDGS on daily feed intake of growing and finishing pigs (g/pig/day)

Treatment	**Period 1**	**Period 2**	**Period 3**	**All**
Age (weeks)	**12**	**15**	**19**	**-**
0	1815	2304	2654	2250
10	1884	2319	2659	2273
20	1790	2236	2538	2175
30	1808	225	2720	2236
P Value	0.122^{NS}	0.321^{NS}	0.095^{NS}	0.082^{NS}
Linear P Value	0.366^{NS}	0.161^{NS}	0.733^{NS}	0.257^{NS}
Quad P Value	0.363^{NS}	0.967^{NS}	0.085^{NS}	0.485^{NS}
sed	39.5	50.5	70.5	37.8
Dev P Value	0.036*	0.219^{NS}	0.064^{TREND}	0.025*

Table 44. Effect of DDGS on daily gain of growing and finishing pigs (g/pig/day)

Treatment	**Period 1**	**Period 2**	**Period 3**	**All**
Age (weeks)	**12**	**15**	**19**	**-**
0	806^{a}	851	917	854^{a}
10	884^{b}	874	921	890^{ab}
20	840^{ac}	825	899	850^{a}
30	872^{bc}	895	940	900^{b}
P Value	0.002**	0.136^{NS}	0.524^{NS}	0.039*
Linear P Value	0.018*	0.391^{NS}	0.589^{NS}	0.140^{NS}
Quad P Value	0.103^{NS}	0.280	0.338	0.614^{NS}
sed	19.6	30.2	27.5	20.0
Dev P Value	0.003**	0.054*	0.316^{NS}	0.014*

Table 45. Effect of DDGS on FCR of growing and finishing pigs

Treatment	Period 1	Period 2	Period 3	All
Age (weeks)	**12**	**15**	**19**	**-**
0	2.26[a]	2.72	2.90	2.62[a]
10	2.14[b]	2.66	2.89	2.56[ab]
20	2.13[b]	2.73[a]	2.84	2.57[ab]
30	2.08[b]	2.53[b]	2.88	2.50[b]
P Value	0.011*	0.045*	0.901[NS]	0.038*
Linear P Value	0.002**	0.040*	0.654[NS]	0.008**
Quad P Value	0.392[NS]	0.197[NS]	0.715[NS]	0.870[NS]
sed	0.051	0.077	0.087	0.040
Dev P Value	0.307[NS]	0.109[NS]	0.632[NS]	0.243[NS]

Table 46. Effect of DDGS on the slaughter and post slaughter characteristics of pigs

DDGS Inclusion	Liveweight (kg)	Hot weight (kg)	P2 (mm)	*Kill out %	Lean meat %	Cold Weight (kg)	Drip Loss %	pH45	pH24
0	99.7[a]	77.16[a]	10.19	77.42	61.96	75.62[a]	0.250	6.576[a]	5.522
10	101.7[bc]	79.34	10.66	77.92	61.66	77.75	0.422	6.621	5.508
20	99.9[ca]	77.13[a]	10.56	77.36	61.61	75.59[a]	0.263	6.591[a]	5.528
30	102.2[b]	79.29	10.56	77.53	61.75	77.71	0.268	6.576[a]	5.509
S.E.D	0.99	0.772	0.1930	0.2306		0.757	0.1773	0.1743	0.2303
P	0.021[**]	0.001[***]	0.080[TREND]	0.068[TREND]		0.001[***]	0.739[NS]	0.973[NS]	0.651[NS]
P (Lin)	0.066[NS]	0.083[NS]	0.109[NS]	0.741[NS]		0.083[NS]	0.839[NS]	0.645[NS]	0.448[NS]
P (Quad)	0.795[NS]	0.959[NS]	0.084[NS]	0.304[NS]		0.959[NS]	0.501[NS]	0.055 TREND	0.490[NS]
CV%	9.3	9.4	17.5	2.8		9.4	560.6	2.64	4.18

*KO% calculated using P2 and cold weight as a covariate. Cold weight has a significant effect of <.001 on KO%, P2 is N.S. at 0.592.

NB. Liveweight at slaughter is different in Table 46 from previous tables. This is due to the data in Table 46 being calculated from the abattoir trial, only clean slaughter pigs were included in the abattoir calculations.

4.5. Summary

The programme was designed to examine the nutritional value of wheat distillers dark grains with solubles (W-DDGS) in poultry and pigs. Nine separate trials were undertaken based on a range of objectives / methodologies. Each trial is associated with a separate report and these are to be read in conjunction with this summary.

Poultry

An initial broiler growth trial up to 28 days of age **(Nottingham)** revealed no significant differences were detected between treatments in terms of performance during both starter and grower phases; no starter x grower interactions were obtained indicating that feeding W-DDGS during the star ter phase did not lead to any adaptation during the grower phase. When performance was assessed over the entire trial (data for starter and grower were combined), there was evidence that birds fed 5% W-DDGS in the starter experienced an inferior FCR overall with increasing W-DDGS in the grower. Comments received from commercial colleagues during initial reporting were that, although differences were not, generally, statistically significant, numerical changes between treatments would be of some considerable importance in a production context. This is a common observation when attempting to reconcile statistical with commercial validity of data. A reduction in the coefficient of apparent ileal nitrogen and amino acid digestibility was observed with increasing levels of W-DDGS. SID values for the W-DDGS studied may need to be lowered. In view of uncertainties over amino acid digestibility (the current trial had used 'assumed' values as directed by Defra LINK), this was examined in a subsequent trial.

The next trial **(Nottingham)** reported values for apparent ileal digestibility (AID) and standard ileal digestibility (SID) of amino acids were similar to those reported elsewhere in the literature, although SID values for lysine were particularly low, being 0.26, 0.27 or 0.32, measured in semi-synthetic, maize or wheat diet backgrounds, respectively. It appeared that diet type employed was influential in the values obtained. The SID values for methionine, cysteine, methionine plus cysteine and arginine were significantly lower ($P < 0.05$) when measured in semi-synthetic diet backgrounds than wheat or corn-based diets. It does appear that dextrose and possibly purified starch have a detrimental impact on the broiler digestive tract. This may impact upon all digestibility methodologies where such a diet base is used.

The two preliminary broiler trials were then followed by a large-scale commercial trial **(H2S)**. This revealed that there were no differences in liveweight, but better Feed Conversion Ratio with W-DDGS although these diets were more expensive as a result of having to include higher levels of pure amino acids; however cost /kg gain was lower and Production Efficiency Factor (PEF) higher. Hock marking and pododermatitis were lower in W-DDGS-based diets It is difficult to draw absolute

conclusions from 1 commercial trial, however the trial has shown that the addition of up to 10% W-DDGS into a balanced broiler diet, had no detrimental effects on the technical performance of the birds. The concerns of the effects that W-DDGS may have on litter quality were not shown in the trial work. More commercial trials need to be carried out to further back up the initial work. The findings did suggest that levels of W-DDGS up to a level of 10%, combined with the use of additional Amino Acids, could provide an alternative protein source in broiler diets.

The programme then considered layers. An initial trial **(Nottingham)** reported that including W-DDGS at up to 18% in diets that were isoenergetic and balanced for digestible amino acids had no effect on performance and egg shell quality; there were no effects of treatment on gut environment / microflora.

The next commercial layer trial **(Noble)** reported that, with an inclusion of 7.5% W-DDGS with the nutritional matrix values ascribed to the raw material in the formulations by Premier Nutrition, there was no practical difference between the trial and control flocks. In addition and in particular concerns over potential increased seconds from using W-DDGS were not realised. W-DDGS therefore can be safely used in layer diets, in part substituting for imported soya. Whether it is actually used or not will depend on the relative values of the product and other raw materials used in least cost formulated layer diets. However, at recent market values it would not feature in a typical layer diet. The W-DDGS was supplied as produced at the plant in a non-pelleted format. In this state the material has a low bulk density which made transport costs excessive due to very poor load factors. In addition it was a difficult material to handle at the mill with significantly increased unloading time. In practice for the material to be of interest to the layer sector it would need to be provided in a pelleted format for milling and transport efficiency reasons.

Pigs

The initial pig trial **(Nottingham)** was an additional programme outside of the initial contract designed to examine amino acid digestibility. The data for standardised ileal digestibility of W-DDGS are not considered sufficiently robust to allow formulation for the subsequent growth trial. Possible reasons for this are based on excessive shedding of mucosal cells giving rise to very large endogenous losses thus resulting in very low or even negative coefficients of digestibility. Such losses could be linked to the effects of semi-purified diets based on starch and glucose and / or electrical stunning during slaughter. Coefficients of Apparent Total Tract Digestibilities of Gross Energy, Phosphorus and Neutral Detergent Fibre were determined following collection of faeces prior to slaughter and ranged from 0.51-0.64, 0.21-0.32 and 0.70-0.77 respectively.

An experiment was conducted **(Illinois, USA)** to determine the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of amino acids (AA) by growing pigs fitted with T-cannulae

in five different sources of DDGS from Europe. The five sources of DDGS include New wheat-maize DDGS, Old wheat DDGS (2012 harvest), New crop UK DDGS (2013 harvest), Hungary maize DDGS, and Germany DDGS. Hungary maize DDGS had the greatest ($P < 0.05$) AID and SID for most AA, but Germany DDGS had the lowest ($P < 0.05$) AID and SID for most AA among all five ingredients. New wheat-maize DDGS, Old wheat DDGS, New crop DDGS, and Germany DDGS had relatively low Lys:CP and AID and SID of Lys compared with Hungary DDGS, which indicates these ingredients may have been heat damaged.

Using data from the Illinois trial, diets were formulated to be iso-energetic and balanced for SID amino acids in a preliminary growth trial **(Nottingham)**. conclusion growing / finishing pigs are able to tolerate levels of W-DDGS up to 300g/kg in pelleted balanced diets in terms of performance and carcass quality (including pH changes post mortem and shoulder fat skatole levels) without a significant reduction in performance. On a 'practical' level, there was a strong trend for a quadratic effect (P=0.063 and 0.058 for grower and finisher; overall no effect, P=0.202) of rate of inclusion of W-DDGS on DLWG, suggesting that a maximum of 200g/kg would be suitable. There were no similar trends for FI and FCR.

In a final commercial growth trial **(Tulip, Harper Adams)**, the inclusion of Wheat Dried Distillers Grains (W-DDGS) at any of the levels in the pelleted diets did not have any negative effects for on farm performance, slaughter characteristics or meat quality. The only significant relationship within the dose response range and structure was FCR in the first two periods with a linear response. All other significant treatment differences could not be explained as part of a dose response. Treatment significances showed early growth differences that affected the overall results. This seemed to be related to early gains in feed efficiency, possibly indicating better gut health and function. No significant treatment effects on P2 corroborate the non-significant performance results in the later periods. It is interesting to note that the zero control showed poorer performance than the other treatment levels both on farm and in the abattoir. The highest inclusion at 30% showed best performance in a number of areas including daily liveweight gain, FCR and slaughter weight. It can therefore be concluded that feeding pigs during the growing and finishing stages with up to 30% W-DDGS included in the diets is an acceptable level. It may also be argued that a future trial may wish to look at a higher inclusion level to determine the optimum inclusion. This trial does not take into account the cost of production and at what inclusion level the W-DDGS would be most economically beneficial.

Comments on diet formulation (from Premier Nutrition)

Least Cost Formulations for Pigs using 2013 and 2014 Cost Sets.

The following formulations were least cost formulated using the raw material prices below. Formulation minimums were set for NE, Digestible phosphorus, digestible lysine and each essential digestible amino acids:digestible lysine ratio for each diet. A minimum for NDF was also set but setting a minimum for NDF is not normal practice in pig diets. Without the minimum NDF level, wDDGS does not feature in the diets and it is not cost effective.

The minimum NDF was the only way that wDDGS would be brought into the formulation when using a cost of £220/t. In the majority of diets, wDDGS only came in when using the 2013 cost set where raw materials were more expensive. The exception is the dry sow diet where it came in for both 2013 and 2014 cost sets. Therefore, even with the minimum NDF level, once the raw material prices become less expensive i.e. 2014 cost set, the wDDGS was not cost effective in pig diets.

Current Cost Sensitivity

If the wDDGS was priced at £0/t in the least cost formulation, it would be included at its 30% maximum inclusion. For wDDGS to compete against rapeseed and wheatfeed using the 2014 cost set in the grower diet, it would need to be £184/t for a 16% inclusion and £120/t or less for a 30% inclusion. For it to compete against these two raw materials in the finisher 60-90kg diet, wDDGS would have to be valued at £110/t or less to achieve a 30% inclusion. £111/t allows a 6% inclusion. These values are not set in stone but are dependent on the nutrient specification used to formulate the diets and the cost of the other raw materials offered to the formulation.

Other factors

For some of the diets where wDDGS has come in, the crude protein and digestible phosphorus has increased. This must be accounted for in the producers NVZ calculations.

Raw material prices used in formulation comparison (£/t)

Raw material	**2014**	**2013**
wheat	117	163
barley	115	143
wheatfeed 8.5% CF	105	148
rapeseed	171	205
sunflower 29% CP	188	210
Hipro soya	320	389
dicalcium phosphate	560	530
wDDGS	220	220
soya oil	750	870
limestone	58	50
salt	116	110
lysine	1500	1250
methionine	4600	2710
threonine	3450	1550
tryptophan	13750	25520
vits and mins	0	0
phytase	5600	5600

5. Ruminant nutritional and performance studies

5.1. Introduction

The main purpose of the ENBBIO ruminant studies was to evaluate Wheat DDGS (wDDGS) from UK bioethanol production in terms of nutritional value and animal responses to inclusion in typical ruminant diets. Although there was an established market for wDDGS in the UK, nutritional values used in diet formulations were based on estimates extrapolated either from imported products or from DDGS produced by the UK whisky industry. In view of the potential tonnage of wDDGS to be derived from UK bioethanol production, there was a need for more accurate evaluation of this product under UK conditions.

A series of trials was conducted to evaluate wDDGS for ruminants and address the following tasks specified in the final LINK Proposal:

Task 2.3 Production responses by dairy cows
Task 4.8 Improving nutrient utilisation by ruminants
Task 4.9 Production responses by dairy cows
Task 4.10 Studies of methane emissions
Task 4.11 Commercial studies – ruminants

There is some overlap between trials and tasks so, for clarity, trials are reported as follows:

Dairy Production Trial 1 (Tasks 2.3 and 4.10)
Sheep ME Trials (Task 4.8)
Rumen Trials (Task 4.8)
Digestibility studies (Task 4.8)
Respiration Chamber Trials (Task 4.10)
Dairy Production Trial 2 (Tasks 4.9 and 4.10)
Beef Commercial Study (Task 4.11)

5.2. Dairy Production Trial 1

Effect of inclusion level of wheat DDGS in the diet on performance and methane emissions by lactating dairy cows.

5.2.1. Objectives

To find the limits at which wDDGS inclusion impairs dry matter intake (DMI) or performance
To quantify the effect of wDDGS on methane emissions by dairy cows.

5.2.2. Background

The aim was to investigate responses to inclusion level of wDDGS in diets for dairy cows. Specifically, wDDGS from UK bioethanol production were tested and responses of primary interest were; dry matter intake, milk yield and methane emissions. The literature, based on maize DDGS and overseas wDDGS, suggests that performance is depressed by wDDGS inclusion levels above 40% of concentrates or 20-30% of total diet DM. This trial aimed to test this hypothesis where wDDGS of UK origin are included in the diet of lactating dairy cows.

5.2.3. Material and Methods

Design

The trial design involved 4 levels of wDDGS inclusion in a Latin square with 4 weeks per treatment period, giving a total of 16 weeks. Each treatment period consisted of 2 weeks diet adaptation and 2 weeks recording. Levels of wDDGS inclusion were 0, 8, 16 and 24% of DMI. Replication was at least 10 cows per level per period. A pre-trial period of two weeks was allowed, during which all cows were fed on a diet with 12% wDDGS inclusion. Cows were blocked into two groups according to stage of lactation (16 early [Group Hi] and 28 mid to late lactation [Group Lo]) and allocated to the 4 initial treatment groups according to milk yield during the second week of the pre-trial period.

Animals, housing and feeding

Cows were all Holstein-Friesians from the Nottingham University Dairy Centre (average annual milk yield 11,400 l/cow). Cows had to be a minimum of five weeks into lactation at selection, and unlikely to be dried-off before the end of the eighteen week study (based on current milk yield and expected calving date). Lame animals were excluded.

Animals were group-housed in a single cubicle yard. Cows in this study group had unrestricted access to a robotic milking system, comprising of a single Lely A3 Astronaut (Lely UK Ltd, St Neots, UK). Cows received concentrates during milking, with a rate of feeding of 3.5kg flat per cow per day, and an additional 0.45 kg/l above 35 l of milk produced for cows in early lactation (Hi group), and above 30 l of milk produced for cows in mid to late lactation (Lo group).

Cows had *ad libitum* access to partial mixed rations (PMR) on an individual basis through Roughage Intake Control (RIC) Feeders (Fullwood Ltd, Ellesmere, UK), and *ad libitum* access to water through six water troughs situated within the housing area. Diets were mixed and fed out using an automated mixing and feeding system (Mix Feeder and Smart Feeder, Mullerup, Ullerslev, Denmark), with fresh feed delivered between the hours of 07:30 and 15:30. Refusals were removed from the RIC Feeders each morning.

Sampling and recording

Samples of all feed ingredients were taken at the end of each feeding period and frozen for later analysis. Milk samples were collected during the final week of each feeding period for determination of fat, protein, lactose and urea. Blood samples were collected during the final week of each feeding period for metabolic profiling.

Individual feed intakes were recorded automatically for each animal through the RIC Feeder system. At each milking animal ID, milk yield and live weight were recorded. Rumination and activity data were recorded by a Lely Qwes-HR tag mounted on the neck collar of each cow and downloaded as 2-hourly means at each milking.

Methane emissions were recorded automatically during each milking using the online monitoring system developed at Nottingham. This involved continuous sampling of air from the feed bin of the robot milker using an infrared methane analyser (Guardian Plus; Edinburgh Instruments Ltd, Livingston, UK) for determination of methane concentrations at one-second intervals. Daily methane emissions were then estimated from frequency of eructations and their methane concentrations, which had been calibrated against data from respiration chambers (Garnsworthy *et al.*, 2012).

Diet Formulation

Diets were formulated to contain wDDGS at 0, 8, 16 and 24% of the total diet on a DM basis. Diets were designed to supply requirements for M+35 for early-lactation cows and M+30 for mid/late lactation cows using a low protein (16%) cake in the robot. For each stage of lactation, two diets were formulated to include 0 and 24% wDDGS – the middle two treatments were produced as composites of these two extremes. All treatment diets contained approximately 50% forage DM supplied by grass, maize and whole-crop silages in proportions 14:13:10. For periods three and four, chopped straw was added to the PMR at the rate of 0.5 kg/cow/day to aid rumination. The concentrate portion of the Control PMR (0% wDDGS) contained wheat, soya, rape, fat, urea, and a premix containing minerals, vitamins and rumen conditioners; for the 24%-wDDGS PMR, most of the wheat and all of the soya, rape, fat and urea were replaced by wDDGS and some SoyPass to ensure adequate bypass protein supply (Table 47).

Calculations and statistical analysis

All data were calculated as daily means and averaged over weeks three and four of each period for each cow. Milk energy output was calculated as (0.384[BF] + 0.223[P] + 0.199[La] - 0.108) times milk yield, where [BF] is butterfat, [P] is crude protein and [La] is lactose contents of milk in g/kg (AFRC, 1993). Whilst daily weight change was calculated as the difference between the beginning and end of each treatment period divided by 28. Feed conversion efficiency (FCE) was calculated

as milk solids yield (fat + protein + lactose) divided by DMI. Conversion efficiencies for gross energy (GECE) and metabolisable energy (MECE) were calculated as milk energy output divided by GE or ME intake. Lastly, nitrogen use efficiency (NUE) was calculated as total nitrogen output in milk (milk protein/6.25) divided by nitrogen intake (CP intake/6.25). Nitrogen use efficiency for true protein output (NUE-TP) was calculated as (total nitrogen - urea nitrogen in milk) divided by nitrogen intake.

Table 47 Formulations of partial mixed rations fed to cows in early (Hi) or mid to late (Lo) lactation and containing four levels of DDGS (values are kg fresh weight per cow per day)

Group	Hi				Lo			
DDGS %	0	8	16	24	0	8	16	24
Grass Silage	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Maize Silage	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Whole Crop silage	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
DDGS	0.000	2.250	4.500	6.750	0.000	2.000	4.000	6.000
Soya/Rape/Fat/Urea blend[1]	5.500	3.667	1.833	0.000	5.000	3.333	1.667	0.000
Wheat-rolled	3.500	2.667	1.833	1.000	2.000	1.333	0.667	0.000
SoyPass	0.000	0.167	0.333	0.500	0.000	0.125	0.250	0.375
Mineral & Vitamin mix	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Sodium bicarbonate	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Limestone Flour	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Biotal Toxisorb	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Biotal Binder	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Ground wheat (Mineral carrier)	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total:	46.9	46.7	46.4	46.2	44.9	44.7	44.5	44.3

For periods 3 and 4, chopped straw was added to each PMR at 0.5 kg/cow/day.

[1]soya-hipro 456, extracted rapeseed 456, Golden Flake fat 49, urea 9, molasses 30 kg/t.

Data were analysed using Genstat (14th Edition). The Residual Maximum Likelihood (REML) procedure was used to fit linear mixed models of the form:

$$y_{ijk} = \mu + Gr + Ls + GLrs + Pi + Cj + \varepsilon_{ijk}$$

Where yijk is the dependent variable; the fixed part of the model consists of

μ the overall constant (grand mean),

Gr the main effect of Group r (where r is the stage of lactation group for unit ijk),

Ls the main effect of DDGS inclusion level s (where s is the level of inclusion for unit ijk), and

GLrs the interaction between Group and Level of inclusion.

The random model terms are;

Pi the effect of Period i,

Cj the effect of Cow j, and

εijk the random error (i.e. residual) for unit ijk.

Data are presented as least-square means predicted by the models for main effects (Group and Level). There was no significant interaction between Group and level for any measurement.

5.2.4. Results

Stage of lactation

Cows in early lactation (Group Hi) had significantly greater intakes of total DM and robot concentrate, greater yields of milk, energy-corrected milk, protein and lactose, higher concentrations of milk fat and urea, and greater live-weight gain, than cows in mid/late lactation (Group Lo) (Table 48).

Table 48 Mean intake, performance and efficiency for cows in early (Hi) or mid/late (Lo) lactation

	Group			
	Hi	Lo	SED	P
DMI, kg/d	23.8	21.9	0.88	**0.032**
PMR DMI, kg/d	17.9	17.1	0.71	0.235
conc DMI, kg/d	6.1	4.9	0.49	**0.025**
Milk yield, kg/d	47.2	37.4	2.26	**<0.001**
ECM yield, kg/d	43.1	36.9	2.00	**0.004**
Fat, kg/d	1.52	1.43	0.093	0.351
Protein, kg/d	1.61	1.27	0.077	**<0.001**
Lactose, kg/d	2.15	1.70	0.104	**<0.001**
Fat, %	3.31	3.86	0.207	**0.010**
Protein, %	3.42	3.40	0.028	0.375
Urea, mg/dl	37.3	34.2	1.50	**0.046**
Lactose, %	4.57	4.53	0.037	0.310
Live weight, kg	671	672	18.13	0.955
LWG, kg/d	0.39	0.20	0.08	**0.016**
BCS	2.55	2.56	0.240	0.974
FCE	0.22	0.20	0.009	**0.037**
NUE	0.35	0.31	0.014	**0.006**

SED, standard error of difference for comparing group means; P, F-ratio probability; DMI, dry matter Intake; PMR, partial-mixed ration; conc, robot concentrate; ECM, energy-corrected milk; LWG, live-weight gain; BCS, body condition score; FCE, feed conversion efficiency; NUE, nitrogen use efficiency.

There was no effect of stage of lactation on plasma albumin, globulin, protein, BOHB or glucose, but Group Hi had significantly higher plasma concentrations of urea-N (5.3 versus 4.9 mmol/l) and NEFA (0.44 versus 0.35 mmol/l) than Group Lo. There was no effect of stage of lactation on daily methane output (mean 361 g/d), methane yield (mean 16.2 g/kg DM) or rumination time (mean 488 min/d).

Level of wDDGS inclusion

When cows were fed on 24% wDDGS they consumed less dry matter, PMR and metabolisable energy than when fed on 0 or 8% wDDGS, but values for 16% wDDGS were not different from other levels (Table 49). Yields of milk, energy-corrected milk, fat, protein and lactose decreased with increasing level of wDDGS inclusion, but differences between treatment means were only significant for the comparison of 0 versus 24% wDDGS. There was no effect of wDDGS inclusion level on milk fat, protein or lactose concentrations, but milk urea concentration was greater for 0 and 8% wDDGS compared with 16 and 24% wDDGS. There was no effect of wDDGS inclusion level on live weight, live-weight gain or body condition score. The only effect of wDDGS inclusion level on efficiency was that the 24% wDDGS inclusion level resulted in a higher efficiency of true protein production than the 0 and 8% levels.

Table 49 Mean intake, performance and efficiency when cows were fed on diets containing 0 to 24% DDGS

	wDDGS inclusion level, %					
	0	8	16	24	SED	P
DMI, kg/d	23.1	23.1	22.9	22.3	0.31	**0.031**
PMR DMI, kg/d	17.7	17.8	17.5	17.0	0.27	**0.025**
conc DMI, kg/d	5.6	5.4	5.6	5.5	0.17	0.586
ME Intake, MJ/d	289	288	287	279	3.9	**0.022**
Milk yield, kg/d	43.6	42.4	41.8	41.4	0.68	**0.012**
ECM yield, kg/d	42.0	40.2	39.0	38.7	0.91	**0.003**
Fat, kg/d	1.58	1.49	1.43	1.41	0.062	**0.021**
Protein, kg/d	1.48	1.44	1.42	1.42	0.023	**0.042**
Lactose, kg/d	1.98	1.93	1.90	1.89	0.032	**0.040**
Fat, %	3.76	3.59	3.50	3.50	0.137	0.130
Protein, %	3.40	3.41	3.41	3.42	0.014	0.262
Urea, mg/dl	38.3	37.0	34.1	33.5	1.04	**<0.001**
Lactose, %	4.54	4.55	4.56	4.56	0.022	0.570
Live weight, kg	674	670	670	672	2.24	0.348
LWG, kg/d	0.29	0.44	0.22	0.23	0.09	0.095
BCS	2.60	2.51	2.56	2.56	0.054	0.234
FCE	0.22	0.21	0.21	0.21	0.004	0.118
NUE	0.34	0.33	0.32	0.33	0.005	0.250
NUE-TP	0.23	0.22	0.23	0.24	0.005	**0.035**

SED, standard error of difference for comparing group means; P, F-ratio probability; DMI, dry matter Intake; PMR, partial-mixed ration; conc, robot concentrate; ME, metabolisable energy; ECM, energy-corrected milk; LWG, live-weight gain; BCS, body condition score; FCE, feed conversion efficiency; NUE, nitrogen use efficiency; NUE-TP, nitrogen use efficiency for true protein synthesis.

Blood urea-nitrogen decreased with increasing inclusion levels of wDDGS. Plasma β-hydroxybutyrate was lower for 0 and 8% wDDGS compared with 16 and 24%. There was no effect of DDGS inclusion level on plasma protein fractions, or NEFA and glucose concentrations (Table 50).

Table 50 Plasma indicators of protein and energy status when cows were fed on diets containing 0 to 24% DDGS

	wDDGS inclusion level, %					
	0	8	16	24	SED	P
Albumin, g/l	26.4	24.7	26.1	26.4	0.97	0.391
Globulin, g/l	27.5	26.3	26.6	26.8	1.04	0.488
Total Protein, g/l	54.0	50.9	52.7	53.1	1.56	0.226
Urea-N, mmol/l	5.4	5.2	5.0	4.8	0.133	**<0.001**
BOHB, mmol/l	0.53	0.52	0.65	0.60	0.034	**<0.001**
NEFA, mmol/l	0.46	0.35	0.39	0.38	0.047	0.394
Glucose, mmol/l	2.56	2.69	2.58	2.67	0.121	0.557

SED, standard error of difference for comparing treatment means; P, F-ratio probability; BOHB, β-hydroxybutyrate; NEFA, non-esterified fatty acids

There was no effect of DDGS inclusion level on daily methane output. When cows were fed on 24% DDGS they produced more methane per kg DMI than when fed on 0 or 8% DDGS, but values for 16% DDGS were not different from other levels. Level of DDGS inclusion did not affect time spent ruminating, but time between boluses was greater for 16 and 24% DDGS compared with 0 and 8% DDGS, and time between chews tended to be lower (P=0.085) for higher levels of inclusion (Table 51).

Table 51 Methane output and rumination parameters when cows were fed on diets containing 0 to 24% wDDGS

	wDDGS inclusion level, %					
	0	8	16	24	SED	P
Methane, g/d	361	359	364	360	2.7	0.262
Methane, g/kg DMI	16.0	15.9	16.3	16.6	0.31	**0.023**
Rumination, min/d	493	493	485	481	8.1	0.314
Bolus interval, s	55.2	55.4	56.0	56.0	0.26	**<0.001**
Chew interval, ms	794	789	789	786	3.1	0.085

SED, standard error of difference for comparing treatment means; P, F-ratio probability

5.2.5. Discussion

Meeting objectives

The first objective of this study was to find the limits to wDDGS inclusion where dry matter intake or performance is impaired. The decreases in dry matter intake and milk yield observed with the highest rate of wDDGS inclusion suggest that under the conditions of this study the limit is between 16 and 24%.

The second objective was to quantify the effect of wDDGS on methane emissions by dairy cows. The lack of effect of wDDGS inclusion on daily methane emissions suggests that, when compared to a control diet that is anticipated to induce low methane emissions, wDDGS does not provide any further potential reduction or increase.

Performance and feed intake

Stage of lactation

Milk yields achieved in this trial were high by UK standards but comparable with those of equivalent cows in the rest of the herd that were fed on the commercial ration. This gives confidence that treatment diets did not impose any overall limitation on performance. As expected, cows in early lactation produced significantly more milk than cows in mid and late lactation, but with a lower fat content. Milk protein and lactose concentrations did not differ with stage of lactation, so yields of these components were significantly higher for cows in early lactation.

Dry matter, energy and nutrient intakes were commensurate with differences in milk yield between groups. A retrospective examination of actual intakes and performance by both groups revealed that metabolisable energy intakes were within 5% of theoretical requirements predicted by Feed into Milk equations (Thomas, 2004). Greater dry matter intake for cows in early lactation was manifested as greater intake of concentrates rather than PMR, which is attributable to the higher concentrate allowance for this group because of their higher milk yield.

Positive changes in live weight were observed throughout the trial period, further suggesting that diets did not limit performance overall. The greater rate of live-weight gain observed for cows in early lactation is probably due to a combination of higher energy density of the diet and a lower starting weight (cows in Group Hi were just past peak milk yield at the start of the trial and would have been at their live-weight nadir).
As expected, cows in early lactation were more efficient in terms of total feed conversion and nitrogen efficiency.

Inclusion of wDDGS
Milk yield and dry matter intake were depressed by the highest level of wDDGS inclusion, although differences were only significant when 24% wDDGS was compared with control. The actual decrease in DMI (0.8 kg/d) was small and was due to reduced intake of PMR rather than concentrates. The difference in milk yield (2.2 l/d) between 0 and 24% wDDGS is in agreement with the difference in metabolisable energy intake (10 MJ/d).

The literature on performance of dairy cows fed on diets containing DDGS is concerned mainly with maize-DDGS; reviewers concur that maize-DDGS can be included at up to 20% of diet DM without depressing intake or performance (e.g. Kalscheur, 2006; Kononoff and Christensen, 2007; Schingoethe *et al.*, 2009), but higher inclusion rates can result in nutritional imbalances. A recent industry review of wheat-DDGS (FOBI, 2011) concluded that feeding wheat-DDGS to dairy cattle "did not negatively affect animal performance and often enhanced milk yield, milk fat yield, and dry matter intake". None of the studies reviewed included DDGS at greater than 20% of diet DM, and enhanced performance was seen only in studies where DDGS replaced barley silage or canola meal. The most recently published study (Chibisa *et al.*, 2012) replaced canola meal with 0, 10, 15 and 20% wheat-DDGS and found no effect on rumen fermentation parameters, but significant increases in dry matter intake and milk yield with DDGS. The authors concluded that "up to 20% wDDGS can be added to lactating cow rations without negatively affecting ruminal function and can potentially increase DM intake and milk yield". Clearly the control diet chosen will influence responses to DDGS; in the current study, the control diet was formulated for optimal performance and, although energy and protein were balanced across diets, the 24% wDDGS diet was sub-optimal for some reason. Whether this is starch content, form of NDF, fatty acid or amino acid profile, or something else has yet to be determined.

Milk fat and protein concentrations did not vary with inclusion level. The protein content of milk is extrapolated from its total nitrogen content, however, which includes nitrogen from true protein (casein, albumin and globulin) and non-protein nitrogen (urea). Milk urea content showed a highly significant decrease with increasing level of wDDGS inclusion, so the linear increase in milk protein

content is due more to enhanced true protein synthesis than to non-protein nitrogen secretion. This resulted in a systematic increase in nitrogen efficiency with increasing wDDGS inclusion when calculated on the basis of true protein output. It is possible that inclusion of urea in the 0% wDDGS diets and soypass in the 24% wDDGS diets contributed to the decrease in milk urea. However, it is also possible that heat treatment of wDDGS could have improved rumen protein degradation, digestibility or metabolisability of key amino acids. Rumen fermentation studies, estimation of digestibility, and amino acid analysis, will provide further information on nitrogen utilisation.

Plasma composition

All plasma metabolites were within their normal range, which gives confidence that none of the diets had a detrimental effect on cow health. Plasma urea was at the high end of the normal range (3 to 5 mmol/l; Ward *et al.*, 1995), which is indicative of excess dietary protein and inefficient capture of rumen degradable protein. This is consistent with the slightly high milk urea concentrations (average = 35, high = 45 mg/dl; Cushnahan, 2003) and the high dietary protein concentrations.

Higher plasma urea and NEFA concentrations for cows in early lactation are normal (Ward *et al.*, 1995). The reduction in plasma urea with increasing wDDGS inclusion level is consistent with the response in milk urea and provides further support for increasing efficiency of nitrogen use on a true-protein basis. Differences between inclusion levels in plasma β-hydroxybutyrate were inconsistent and cannot be explained; all means were, however, well below values that cause concern (0.9 mmol/l; Ward *et al.*, 1995).

Methane emissions

The lack of difference in daily methane emissions between cows in early and mid/late lactation, despite a difference in dry matter intake, can be explained by the higher proportion of concentrates in the diet consumed by cows in early lactation.

It was anticipated that wDDGS might reduce daily methane emissions; work at the Rowett Research Institute (analysed and reported by Giger-Reverdin and Sauvant, 2000) found that DDGS resulted in the lowest methane emissions of all concentrate feed ingredients. Additionally, McGinn *et al.* (2009) reported a 20% decrease in methane emissions when DDGS replaced barley in diets for fattening beef cattle. In the current study, however, there was no effect of wDDGS inclusion level on daily methane emissions. The most likely explanation is that in previous studies the effects of DDGS on methane have been attributed mainly to fat content. For example, in the study of McGinn *et al.* (2009), substitution of DDGS for barley resulted in a 3% increase in dietary fat content. In the current study, supplemental fat was included the control diet so there was little difference in fat content across levels of wDDGS inclusion.

Methane emissions were low in this study; average emissions were 4.5% of gross energy intake, compared with 6.5% used by IPCC calculations for national inventories. Lower emissions are expected from high-yielding dairy cows fed on diets with a high proportion of concentrates (Beauchemin *et al.*, 2009).

The linear trends for increased methane emissions per unit of dry matter intake reflect the reductions in intake with increasing level of wDDGS.

Rumination

On average cows spent approximately one third of each day ruminating, which is normal. Some individuals showed reduced rumination time in the first two periods, which was not related to DDGS inclusion level, and was overcome by addition of straw to all diets in periods 3 and 4. The tendency for cows in early lactation to spend more time ruminating reflects their greater dry matter intake. Decreased rumination time has been reported in studies where wet DDGS has replaced barley silage, but not when dry DDGS has replaced barley grain or canola (FOBI, 2011). Results of the current study support the general consensus that wDDGS do not reduce rumination time when replacing concentrate ingredients.

5.2.6. Conclusions

The main conclusions from this study are:

1. Cows at all stages of lactation showed similar responses to inclusion level of wDDGS.
2. Compared with the control diet, dry matter intake and milk yield were depressed by the highest level of wDDGS inclusion (24%).
3. Level of wDDGS inclusion did not influence major milk constituents, but higher wDDGS levels reduced milk and plasma urea concentrations, thus improving nitrogen efficiency in terms of true protein.
4. Level of wDDGS inclusion did not influence daily methane emissions, which were generally low.
5. The results of this study are in agreement with general literature conclusions that wDDGS can be included in diets for dairy cows at up to 20% of total diet DM without detrimental effect on cow performance or health.

5.3. Sheep ME Trials

Metabolisable energy, digestibility and methane production for DDGS in sheep fed at maintenance

5.3.1. Objectives

To measure ME content of four DDGS samples using the standard technique.

To provide digestibility coefficients for dry matter (DM), nitrogen, oil and gross energy (GE).

To provide estimates of methane production by sheep fed on DDGS at maintenance.

5.3.2. Background

In the first dairy cow study, milk yield was depressed by the highest level of wheat distillers grains (wDDGS) inclusion compared with the control diet. One possible explanation is that the metabolisable energy (ME) content of the wDDGS might be lower than was assumed during diet formulation. The Feed into Milk system uses ME values of feed materials determined in sheep fed at maintenance, which are scaled up for lactating dairy cows. No sheep ME value is available for wDDGS from UK bioethanol production.

5.3.3. Material and Methods

Two metabolism and methane trials were conducted to evaluate four samples of DDGS. In Trial 1, the wDDGS used in the first dairy cow study (wDDGS-1) was compared with a maize DDGS (mDDGS). In Trial 2, two samples of wDDGS with low (wDDGS-2) and high (wDDGS-3) levels of solubles (syrup) were compared.

Metabolism studies

Four wether sheep (live weight 50-60 kg) were used throughout the studies. Each trial had four phases lasting 26 days each. During the first 16 days of each phase, sheep were housed in individual pens, bedded on hemp; for the remaining 10 days sheep were housed in individual metabolism crates. Throughout each phase, sheep were fed twice daily at approximately 8:30 and 16:00. Water was available *ad libitum* throughout.

Whilst in metabolism crates, any feed refusals were weighed and recorded. Faecal output was weighed twice daily and a subsample of approximately 200g taken for analysis. Urine was collected into a plastic tub and volumes measured twice daily using a measuring cylinder. Evaporation of volatiles such as ammonia was prevented by prior acidification of urine tubs with 20ml of 50% sulphuric acid (H_2SO_4). A subsample of approximately 150 ml was taken twice daily for analysis. Faecal and urine samples were stored at -20°C until analysed.

Phase 1 – all four sheep were fed on chopped grass hay. The hay selected was of good quality in terms of palatability and freedom from mould, but was likely to have low digestibility due to its high stem content. Sufficient hay was prepared to last through all phases. Each sheep was offered 1000 g hay per day divided into two equal meals.

Phase 2 – two sheep were fed on one DDGS plus hay and two were fed on the other DDGS plus hay. Both DDGS were offered at 700 g/d with hay at 700 g/d, split into two equal meals.

Phase 3 – two sheep were fed on each DDGS plus hay. Sheep that were fed on one DDGS in Phase 2 were swapped to the other DDGS and vice versa. Both DDGS were offered at 700 g/d with hay at 700 g/d, split into two equal meals.

Phase 4 – all four sheep were fed on chopped grass hay. Each sheep was offered 1000 g hay per day divided into two equal meals.

Methane studies

The same four sheep were used to determine methane emissions associated with the diets used in the two metabolism trials. For each metabolism trial, methane measurements were made in four periods, each lasting 14 days. During the first 7 days of each period sheep were housed in individual pens in a barn, bedded on hemp; for the remaining 7 days sheep were housed in individual pens within a metabolism room. Throughout each period, sheep were fed twice daily at approximately 8:30 and 16:00. Water was available *ad libitum* throughout.

Period 1 – all four sheep were fed on chopped grass hay. Each sheep was offered 1000 g hay per day divided into two equal meals.

Period 2 – all four sheep were fed on one DDGS (700 g/d) plus hay (700 g/d), split into two equal meals.

Period 3 – all four sheep were fed on the other DDGS (700 g/d) plus hay (700 g/d), split into two equal meals.

Period 4 – all four sheep were fed on chopped grass hay. Each sheep was offered 1000 g hay per day divided into two equal meals.

Methane concentration was measured at the inlet and outlet vents of the metabolism room. Air was sampled alternately from inlet and outlet for periods of two minutes, with methane concentration recorded for the second minute of each period. Methane production in each 4-minute sampling period was calculated as the difference in methane concentration between inlet and outlet multiplied by airflow in the outlet duct. Data for 32 minutes after the door was opened at each feeding time were discarded. Daily methane production was calculated as the average emission rate, expressed as a percentage of daily GE intake.

Laboratory analysis

Samples of feed and faeces were analysed for DM, nitrogen, oil and GE. Urine samples were analysed for nitrogen and GE. Dry matter was determined by oven drying at 80°C until stable

weight (usually around 5 days). Nitrogen was determined by elemental Dumas analyser (Carlo Erba Instruments, NA 2000 nitrogen analyser). Oil was determined by ether extract. Gross energy was determined by bomb calorimeter.

Dry matter and nitrogen analyses were conducted at Nottingham. Oil and GE analyses were conducted by Sciantec.

Samples of each feed were collected at the start of each phase, at 10 days, and at 25 days. Each sample was analysed individually for DM and nitrogen. Samples were pooled for oil and GE analysis.

Samples of faeces were collected at each am and pm weighing when sheep were in metabolism crates (20 samples per sheep per collection period). Each sample was analysed individually for DM and nitrogen. Samples were pooled within day according to weight of faeces at am and pm collections for each sheep (10 samples per sheep per collection period) for oil and GE analysis.

Samples of urine were collected at each am and pm weighing when sheep were in metabolism crates (20 samples per sheep per collection period). Each sample was analysed individually for DM and nitrogen. Samples were pooled within day according to volume of urine at am and pm collections for each sheep (10 samples per sheep per collection period) for oil and GE analysis.

Calculations

Digestibility coefficients for DM, nitrogen, oil and GE were calculated for each day of the collection period as:

Digestibility = (*Intake – Faecal Output*) / *Intake*

Where *Intake* is the sum of feed components offered at am and pm meals minus any refusals, and *Faecal Output* is the sum of faecal components recorded at am and pm weighings.

In phases 2 and 3, faecal output attributed to DDGS was calculated as:

Faecal Output from DDGS = *Total Faecal Output* – [*Hay Intake* x (1 – *Hay Digestibility*)]

Where *Total Faecal Output* is the sum of faecal components recorded at am and pm weighings, *Hay Intake* is the sum of hay components offered at am and pm meals minus any refusals, and *Hay Digestibility* is the average digestibility of hay components in Phases 1 and 4 for each individual sheep.

Metabolisable energy content of feeds was calculated as:

ME (g/kg DM) = (*GE Intake – Faecal GE Output – Urine GE Output – CH_4 GE Output*) / *DMI*

Where *CH_4 GE Output* is methane energy output calculated as *GE Intake* x *Methane Factor*. *Methane Factor* is the percentage of GE intake lost as CH_4 for each diet, measured in a respiration chamber (see later).

In phases 2 and 3, urine output attributed to wDDGS or mDDGS was calculated as:

Urine GE Output from DDGS = *Total Urine GE Output* – (*Hay GE Intake* x *Hay GE Urine*)

Where *Hay GE Urine* is the average proportion of *Hay GE Intake* lost in urine in Phases 1 and 4 for each individual sheep.

Statistical analysis

Data were analysed using Genstat (16th Edition). For the metabolism studies, the Residual Maximum Likelihood (REML) procedure was used to fit linear mixed models of the form:

$y_{ijk} = \mu + F_s + P_i + S_j + D_k + \varepsilon_{ijk}$

where y_{ijk} is the dependent variable;

the fixed part of the model consists of

μ the overall constant (grand mean), F_s the main effect of Feed being tested;

the random model terms are

P_i the effect of Phase i, S_j the effect of Sheep j, D_k the effect of Day within Phase, and

ε_{ijk} the random error (i.e. residual) for unit ijk.

The model was applied to digestibility coefficients for DM, nitrogen, oil and GE, and to DE and ME contents.

For the methane studies, data were analysed using the Repeated Measures Analysis of Variance procedure with DDGS type as the treatment effect and days as the repeated measures.

5.3.4. Results

Metabolism studies

In Trial 1, digestibility of GE was higher for mDDGS than for wDDGS-1, but there was no difference between samples in digestibility of DM, nitrogen or oil (Table 52). Concentration of ME was greater for mDDGS than for wDDGS1.

Table 52 Digestibility coefficients and energy contents of mDDGS and wDDGS-1

	mDDGS	wDDGS-1	SED	P
Digestibility				
DM	0.671	0.649	0.0204	0.290
N	0.720	0.715	0.0133	0.743
Oil	0.887	0.872	0.0169	0.365
GE	0.722	0.676	0.0169	0.008
ME (MJ/kg DM)	14.4	12.1	0.38	<0.001

SED, standard error of difference for comparing treatment means; P, F-ratio probability

In Trial 2, digestibility coefficients for DM, nitrogen and GE were higher for wDDGS-3 than for wDDGS-2, but there was no difference between samples in digestibility of oil (Table 53). Concentration of ME was greater for wDDGS-3 than for wDDGS-2.

Methane studies

In Trial 1, methane output was similar when sheep were fed on hay plus wDDGS-1or hay plus mDDGS (Table 53). For both DDGS diets, methane output was reduced per kg DMI or as a percentage of GEI compared with feeding hay alone.

Table 53 Digestibility coefficients and energy contents of wDDGS-2 and wDDGS-3

	wDDGS-2	wDDGS-3	SED	P
Digestibility				
DM	0.619	0.703	0.0068	0.001
N	0.686	0.733	0.0094	0.016
Oil	0.887	0.856	0.0180	0.185
GE	0.651	0.715	0.0168	0.032
ME (MJ/kg DM)	12.5	13.4	0.19	0.016

SED, standard error of difference for comparing treatment means; P, F-ratio probability

Table 54 Methane output by sheep fed on diets consisting of hay alone or hay plus wDDGS-1 or mDDGS

	Hay alone	Hay + wDDGS-1	Hay + mDDGS	SED	P
CH_4 g/d	11.0	14.9	12.8	1.84	0.376
CH_4 g/kg DMI	12.9	10.7	9.1	1.32	0.341
CH_4 % GEI	4.31	3.01	2.45	0.366	0.265

SED, standard error of difference for comparing DDGS means; P, F-ratio probability of difference between DDGS means

In Trial 2, methane output was similar when sheep were fed on hay plus wDDGS-2 or hay plus wDDGS-3 (Table 55), although there were strong tendencies for wDDGS-2 diets to result in greater methane output than wDDGS-3 diets. For both DDGS diets, methane output was reduced per kg DMI or as a percentage of GEI compared with feeding hay alone.

Table 55 Methane output by sheep fed on diets consisting of hay alone or hay plus wDDGS-2 or wDDGS-3

	Hay alone	Hay + wDDGS-2	Hay + wDDGS-3	SED	P
CH_4 g/d	8.9	16.7	10.1	1.60	0.054
CH_4 g/kg DMI	14.4	11.8	7.2	1.13	0.056
CH_4 % GEI	4.67	3.45	2.17	0.334	0.062

SED, standard error of difference for comparing DDGS means; P, F-ratio probability of difference between DDGS means

5.3.5 Discussion

In Trial 1, mDDGS had a higher ME content than wDDGS-1; in Trial 2, wDDGS-3 had a higher ME content than wDDGS-2. Superiority of mDDGS was expected because the mDDGS sample had a higher oil content than wDDGS-1; superiority of wDDGS-3 was expected because it contained a higher proportion of added solubles.

The Rowett Feedingstuffs Evaluation Unit Fourth Report (Wainman *et al.*, 1984) lists results for five samples of DDGS tested in combination with hay, as in the current study. Three samples were from maize and two from barley. Digestibility coefficients of DM and GE for mDDGS in the current study were within range of the three Rowett mDDGS samples (Figure 22). Digestibility coefficients of DM and GE for the three wDDGS in the current study were similar, on average, to the two Rowett bDDGS samples. ME value for mDDGS was within range of the three Rowett mDDGS samples (Figure 21). ME values for the three wDDGS in the current study were similar, on average, to the two Rowett bDDGS samples.

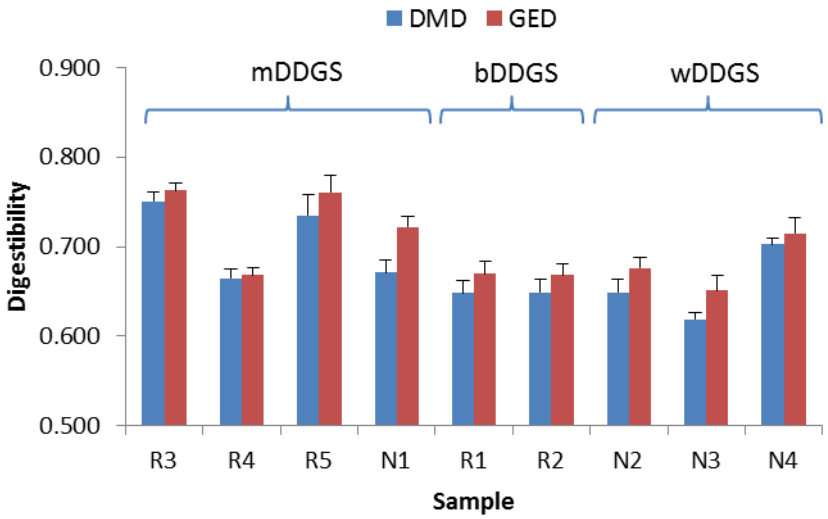


Figure 21 Digestibility coefficients for DM and GE of mDDGS, bDDGS and wDDGS samples tested at the Rowett Institute (R1 – R5) and Nottingham (N1 – N4). N1 is mDDGS; N2 is wDDGS-1; N3 is wDDGS-2; and N4 is wDDGS-3.

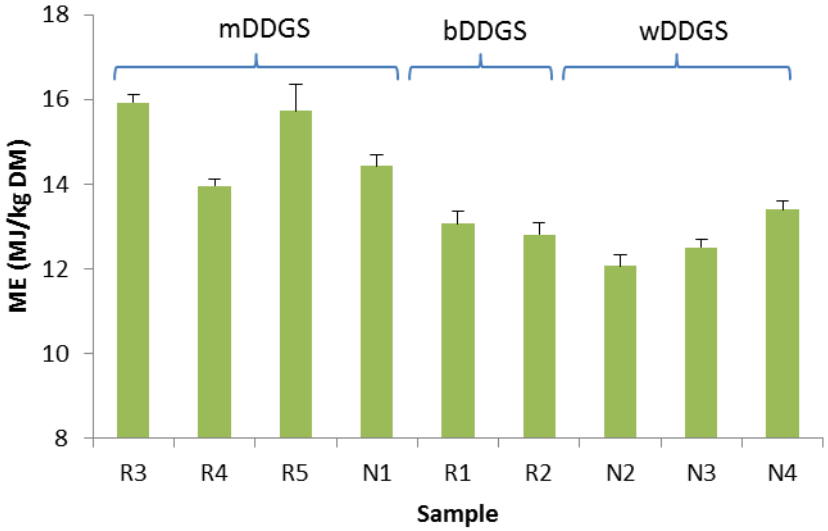


Figure 22 Metabolisable energy content of mDDGS, bDDGS and wDDGS samples tested at the Rowett Institute (R1 – R5) and Nottingham (N1 – N4). N1 is mDDGS; N2 is wDDGS-1; N3 is wDDGS-2; and N4 is wDDGS-3.

Gizzi and Givens (2001) reviewed the use of distillers' dark grains from the whisky industry in ruminant nutrition. Across 21 *in vivo* evaluations, digestibility coefficients ranged from 0.648 to 0.830, and ME concentrations ranged from 12.1 to 15.6 MJ/kg DM. In agreement with the current study, they found higher ME values for mDDGS (mean 15.2 MJ/kg DM) than for wDDGS (mean 13.7 MJ/kg DM). ME values observed in the current study are in agreement with an article on whisky distillers grains in 'The Encyclopaedia of Farm Animal Nutrition' (M. Fuller, ed), which quotes ME values of 14.0 MJ/kg DM for mDDGS and 12.5 MJ/kg DM for wDDGS. For Canadian bioethanol DDGS, Nuez-Ortín and Yu (2009) calculated ME values equivalent to 14.6 for mDDGS and 12.6 for wDDGS.

Methane emissions in the Rowett studies ranged from 2.0 to 2.9 % GEI for mDDGS and 4.8 to 5.0 % GEI for bDDGS, which are of similar order to those observed in the current study.

5.3.6 Conclusions

1. It is concluded that for the feeds tested in this study ME values (MJ/kg DM) are 14.4 ±0.4 for mDDGS, 12.1 ±0.4 for wDDGS-1, 12.5 ±0.2 for wDDGS-2 and 13.4 ±0.2 for wDDGS-3.
2. Values for digestibility, ME and methane emissions are in agreement with the literature.

5.4 Rumen Trials

5.4.1 Objectives

To determine rumen degradability characteristics of dry matter and nitrogen for a range of DDGS samples.

To examine the effect of wDDGS on rumen volatile fatty acid concentrations.

5.4.2 Background

In the first dairy cow study, milk yield was depressed by the highest level of wheat distillers grains (wDDGS) inclusion compared with the control diet. One possible explanation is that rumen degradability characteristics of the wDDGS might be different to those assumed during diet formulation, resulting in lowered effective rumen degradable protein (ERDP) or metabolisable protein (MP) supply. The Feed into Milk system calculates ERDP and MP supplies from dietary crude protein content and the degradability characteristics of dry matter and nitrogen in dietary ingredients. Because a large proportion of dietary nitrogen was supplied by wDDGS in the diet with highest wDDGS inclusion, it was important to determine degradability characteristics of this ingredient, and also a range of other DDGS samples for comparison.

Different carbohydrate sources can lead to different profiles of volatile fatty acids (VFA) in the rumen; starchy concentrates favour propionate and fibrous concentrates favour acetate. Changes in rumen VFA profiles can influence milk yield and composition. Because the ratio of dietary starch to fibre concentrations decreased with increasing wDDGS inclusion level, it was desirable to see if this affected rumen VFA profiles.

5.4.3 Material and Methods

Animals and feeding

Two non-lactating adult Holstein cows fitted with rumen cannulae were used in these studies. For degradability studies they were fed on a diet of grass hay (*ad libitum*) and a commercial concentrate (1 kg/d). For the VFA study they were fed on diets from Dairy Production Trial 1 containing 0 or 24% wDDGS-1 at the maintenance level (fresh weight 20 kg/d).

Degradability studies

Degradability characteristics were determined for a total of 22 DDGS samples. Samples w1-4 were wDDGS from UK bioethanol production (w1 is wDDGS-1, w2 is wDDGS-2, and w3 is wDDGS-3 in dairy and sheep trials); wF was wDDGS from France; wm1 was a wheat/maize DDGS mixture from UK; wmG was a wheat/maize DDGS mixture from Germany; m1 was a mDDGS from UK (mDDGS in sheep trial 1); mH was a mDDGS from Hungary. The remaining 13 samples were from an extrusion study and results are reported in the processing section (Section 7).

For each sample, approximately 20g of DDGS was weighed accurately into each of 28 Dacron bags. Fourteen bags were inserted into the rumen of each cow and removed after 0, 4, 8, 12, 24, 48 or 72 hours (two bags per cow per incubation time). After removal Dacron bags were rinsed immediately under running water and then washed in a domestic washing machine for 20 minutes

at 30°C. Bags were then placed in an oven at 80°C for a minimum of 48 hours or until a constant weight was achieved on two consecutive days. Residues remaining in the Dacron bags were analysed for nitrogen content using an elemental analyser (Dumas method). Concentration and aqueous solubility of dry matter (DM) and nitrogen in the original samples were also determined by repeated washing and filtering through a Whatman 541 filter paper.

Loss of DM and nitrogen from bags for each sample was fitted by non-linear regression to the model:

$D = a + b(1 - e^{-ct})$

where D = disappearance of DM or N at time *t*, *a* is the intercept which represents the rapidly soluble fraction, *b* is the asymptote which represents the potentially degradable fraction, and *c* is the exponential rate of degradation.

Effective degradability was calculated using the equation

$$\text{Effective degradability} = a + \frac{bc}{(c+k)}$$

where *k* is the fractional outflow from the rumen, assumed for dairy cows to be 0.08 (8% per hour).

Rumen VFA studies

Each of two cows was fed on a diet from Dairy Production Trial 1 containing 0 or 24% wDDGS-1 at the maintenance level (fresh weight 20 kg/d) for 10 days. Daily feed allowances were divided into two equal portions offered at 08:00 and 16:00 each day. On the tenth day, samples of rumen fluid (approximately 50 ml) were collected every hour between 08:00 and 21:00. Each cow was then transferred to the other diet and the regimen repeated.

After collection of each sample, pH was measured immediately. A 10ml aliquot of each sample was then centrifuged (4000 rpm at room temperature) for 15mins. One ml of supernatant was mixed with 0.2 ml 25% metaphosphoric acid and incubated at room temperature for 30 minutes before being frozen at -20 °C until needed for analysis.

Volatile fatty acid concentrations were determined by gas chromatography using the method of Playne, 1985.

5.4.4 Results

Degradability characteristics of the 9 samples varied markedly (Table 56), but there was no consistent difference between DDGS from wheat, maize or mixtures. Degradability of DM and nitrogen were highly correlated (r^2 = 0.70) and reflected differences in the rapidly soluble (a)

fraction (r^2 = 0.60 for DM and 0.64 for N). However, the rapidly soluble (a) fraction was not correlated with aqueous solubility (r^2 = 0.03 for DM and 0.01 for N).

Table 56 Degradability characteristics (%) of 9 samples of DDGS

	Wheat					*Wheat & Maize*		*Maize*	
	w1	w2	w3	w4	wF	wm1	wmG	m1	mH
Dry matter									
a	51.0	31.5	33.4	40.5	39.8	42.9	59.2	52.7	44.4
b	36.1	24.9	40.2	35.4	47.1	50.6	24.5	47.2	33.2
c	0.044	0.065	0.100	0.034	0.093	0.023	0.054	0.015	0.035
Degradability	63.8	42.6	55.7	51.1	65.1	54.3	69.0	60.3	54.6
Solubility	35.2	30.3	33.5	32.3	31.8	32.4	35.6	27.6	25.4
Nitrogen									
a	23.6	16.9	31.1	21.1	31.6	22.8	49.6	42.5	29.7
b	67.0	25.5	46.4	75.3	60.3	90.8	30.8	36.3	29.4
c	0.039	0.046	0.099	0.037	0.060	0.015	0.057	0.029	0.049
Degradability	45.5	26.2	56.8	44.8	57.3	36.9	62.4	52.0	40.8
Solubility	18.5	16.1	25.5	12.5	11.4	21.5	21.4	12.9	15.3

Key to DDGS origins: w1-w4 = UK wheat; wF = France wheat; wm1 = UK wheat/maize mixture; wmG = Germany wheat/maize mixture; m1 = UK maize; mH = Hungary maize.

Rumen pH showed significant (P=0.038) diurnal variation (Figure 23), being highest at feeding times and lowest approximately 5 hours after feeding. There was no interaction between diet and time, but pH was significantly lower (P=0.019) when cows were fed on the diet containing 24% wDDGS-1 (mean 6.20) than when they were fed on the diet containing 0% wDDGS-1 (mean 6.33).

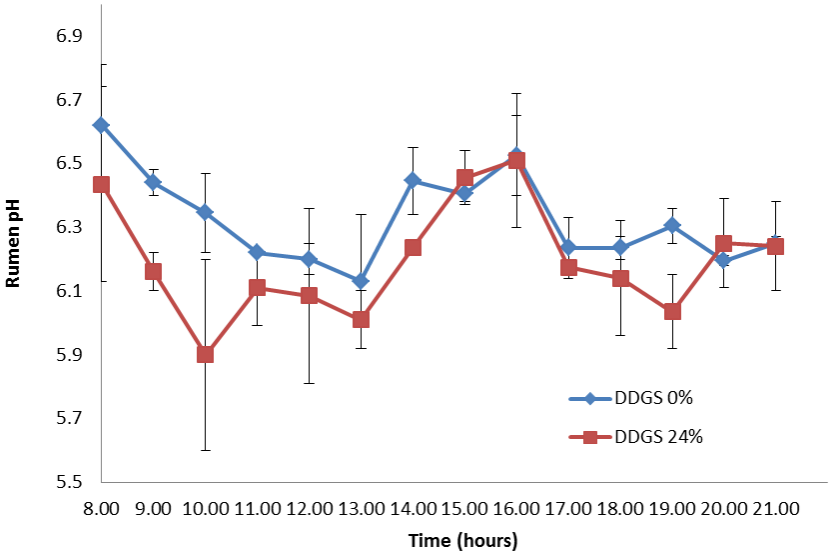


Figure 23 Diurnal variation in rumen pH for cows fed on diets containing 0 or 24% wDDGS-1.

Rumen acetate proportion showed significant (P=0.025) diurnal variation (Figure 24), being highest at feeding times and lowest 3 to 4 hours after feeding. Rumen propionate and butyrate proportions did not vary with time of day.

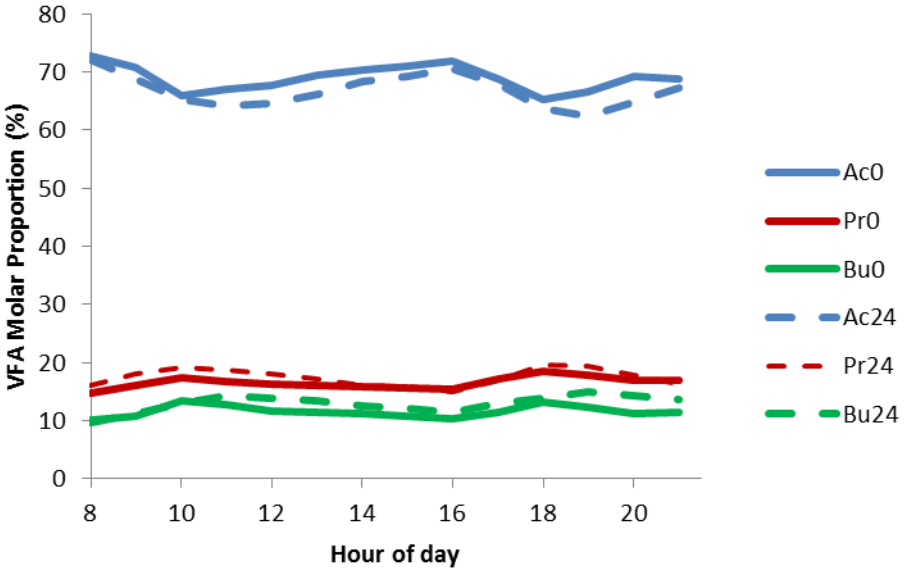


Figure 24 Diurnal variation in rumen acetate (Ac), propionate (Pr) and butyrate (Bu) proportions for cows fed on diets containing 0 or 24% wDDGS-1.

Proportions of acetate and valerate were significantly lower, and proportions of propionate and butyrate were significantly higher, when cows were fed on the diet containing 24% wDDGS-1 than when they were fed on the control diet (Table 57).

Table 57 Molar proportions (%) of volatile fatty acids in rumen fluid from cows fed on diets containing 0 or 24% wDDGS-1

	DDGS inclusion level			
	0	24	SED	P
Acetate	68.9	66.8	0.85	0.018
Propionate	16.5	17.4	0.45	0.050
Butyrate	11.5	12.9	0.52	0.012
Isobutyrate	0.72	0.70	0.052	0.568
Isovalerate	1.43	1.49	0.066	0.345
Valerate	0.91	0.70	0.088	0.025

5.4.5 Discussion

Degradability characteristics

Nitrogen degradability characteristics of the DDGS samples tested are comparable with the widely varying values reported by Westreicher-Kristen *et al.* (2012) for DDGS samples from European ethanol plants; for wDDGS they found a range of 10.2 to 21.1% for the 'a' fraction, 60.9 to 72.2%

for the ‘b’ fraction, and 5.2 to 37.8% for the rate of degradation (‘c’); for mDDGS, ranges were 17.0 to 19.1% for ‘a’, 66.3 to 82.7% for ‘b’, and 2.7 to 4.5 for ‘c’. Nuez-Ortín and Yu (2009) reported 24-hour degradability values of Canadian wDDGS to be 68% for dry matter and 42% for nitrogen; although not directly comparable due to methodology, mean 24-hour degradability values of UK wDDGS were 68% for dry matter and 59% for nitrogen. In the review of distillers’ dark grains from the whisky industry by Gizzi and Givens (2001), nitrogen degradability characteristics ranged from 22.6 to 87.0% for the ‘a’ fraction, 10.6 to 87.1% for the ‘b’ fraction, and 2.6 to 17.0% for the rate of degradation (‘c’). Thus, findings of the current study support conclusions from the literature that degradability characteristics of dry matter and nitrogen in DDGS show considerable variation.

In view of the variation and implications of degradability characteristics for ERDP and MP supplies, it is important that these characteristics are established for different types of DDGS. The main sources of variation are likely to be the proportion of solubles blended with the grains and the degree of heating during the drying process. These factors were not known in the current study, or in the literature, but we do know that w3 had a greater proportion of solubles than w1, w2 or w4. This is reflected in higher values for rapidly soluble nitrogen (‘a’ fraction) and rate of degradation (‘c’) for w3. Sample w3 also had the highest nitrogen solubility value, although rapidly soluble nitrogen and nitrogen solubility were not correlated across all samples. This is because the ‘rapidly soluble’ fraction includes small particles that wash out of Dacron bags and are not necessarily soluble. More research is needed, but the results suggest that 64% of variation in nitrogen degradability may be accounted for by determining the rapidly soluble fraction, which could provide a useful laboratory method to adjust for differences in the proportion of solubles included in batches of DDGS.

Rumen fermentation

Rumen pH followed the classic pattern of diurnal variation observed in animals fed twice daily; pH decreased for approximately 5 hours after each feeding time as fermentable carbohydrates were converted to VFA, and then increased as rumination and chewing of long fibre encouraged saliva production and rumen buffering. Although mean rumen pH was lower when cows were fed on the diet containing 25% wDDGS-1 than when they were fed on the control diet, at no time did pH drop below the value of 5.5 considered critical for sub-acute ruminal acidosis (Kleen *et al.*, 2003).

Differences between diets in molar proportions of VFA, although significant, were small and unlikely to be of practical significance in affecting milk yield or composition. Propionate is a precursor of blood glucose, which could favour either increased milk synthesis or body fat deposition. Acetate and butyrate are both precursors for de novo mammary synthesis of butterfat, so the decrease in proportion of acetate would be offset by the increase in proportion of butyrate. Previous studies have reported no effect of wDDGS on rumen pH or molar proportions of VFA when replacing barley silage and grain (Li *et al.*, 2011) or barley grain (Walter *et al.*, 2012) in feedlot diets for beef heifers.

5.4.6 Conclusions

1. Degradability characteristics of DDGS have a profound effect on calculated supplies of ERDP and MP, which can make the difference between deficiency and surplus in diet formulation.
2. Degradability characteristics of DDGS vary markedly, probably as a result of proportion of solubles added and heat treatment during drying.
3. Determination of the rapidly soluble fraction of DDGS samples might provide sufficient information to allow for differences in proportion of solubles and effective degradability.
4. Rumen pH and proportions of volatile fatty acids were affected by inclusion of wDDGS, but these effects are unlikely to be of practical significance.

5.5 Digestibility Trials

5.5.1 Objective

To investigate the effect of DDGS inclusion level on whole-diet digestibility in high yielding dairy cows.

5.5.2 Background

In the first dairy cow study, milk yield was depressed by the highest level of wheat distillers grains (wDDGS) inclusion compared with the control diet. One possible explanation is that digestibility of the diet, or the protein fraction of the diet, might be reduced when DDGS are included in the diet.
Digestibility can be measured by total faeces collection from animals housed individually in pens or standings, but this is labour intensive and can disrupt normal feeding behaviour. An alternative approach is to calculate digestibility from concentrations of inert markers in feed and faeces; because digestible substances are removed from feed as it passes through the gut, concentration of an inert marker should be proportionally higher in faeces than in feed. Acid-insoluble ash is an inert market that has been shown to provide good agreement with total faecal collection (Van Keulen and Young, 1977).

5.5.3 Material and Methods

Animals and Treatments

These are described under Dairy Trial 1. Briefly, four levels of DDGS inclusion (0, 8, 16, 24%) were incorporated into diets for 44 high-yielding dairy cows in early (Hi; n=16) or mid/late lactation (Lo; n=28), and fed in a Latin square design with 4 weeks per treatment period.

Faeces collection and analysis

One sample (approximately 150ml) of freshly-voided faeces was collected from each cow during the last four days of each of treatment periods 3 and 4. The pen of cows was observed daily between the hours of 08:00 and 17:00 for signs of imminent defaecation. Whenever possible, voided faeces were collected into a bucket held behind the cow. Alternatively, a sample was taken

from a freshly produced dung pat, taking care to avoid contamination by bedding or slurry. Samples were sealed into a plastic bag and stored at -20°C prior to analysis.

Feed and faeces samples were dried in an oven at 75°C until constant weight (2-3 days). Nitrogen content was determined by elemental analysis (Dumas method). Acid-insoluble ash (AIA) was determined by the method of Van Keulen and Young (1977).

Digestibility calculation

DM digestibility was calculated using the formula:

Digestibility = 1- ({g AIA/kg diet DM}) / {g AIA/kg faeces DM})

Nitrogen digestibility was calculated using the formula:

N digestibility = 1 – [({g N/kg faeces DM} x {g AIA/kg diet DM}) / ({g N/kg diet DM} x {g AIA/kg faeces DM})]

Statistical Analysis

Data were analysed using Genstat (14th Edition). The Residual Maximum Likelihood (REML) procedure was used to fit linear mixed models of the form:

$y_{ijk} = \mu + G_r + L_s + GL_{rs} + P_i + C_j + \varepsilon_{ijk}$

where y_{ijk} is the dependent variable;

the *fixed* part of the model consists of

μ the overall constant (grand mean),

G_r the main effect of Group r (where r is the stage of lactation group for unit ijk),

L_s the main effect of DDGS inclusion level s (where s is the level of inclusion for unit ijk), and

GL_{rs} the interaction between Group and Level of inclusion;

the *random* model terms are

P_i the effect of Period i,

C_j the effect of Cow j, and

ε_{ijk} the random error (i.e. residual) for unit ijk.

5.5.4 Results

There was no significant effect of stage of lactation on digestibility of dry matter or nitrogen (Table 58). Inclusion level of DDGS did not affect digestibility of dry matter, but nitrogen digestibility was significantly lower for the 16 and 24% inclusion levels than for the 0 and 8% inclusion levels (Table 59). There was no interaction between stage of lactation and inclusion level for digestibility of dry matter or nitrogen (Table 60).

Table 58 Digestibility of dry matter (DM) and nitrogen (N) for cows in early (Hi) or mid/late (Lo) lactation

	Group			
	Hi	Lo	SED	P
Dry Matter	0.712	0.706	0.009	0.516
Nitrogen	0.696	0.688	0.010	0.438

Table 59 Digestibility of dry matter (DM) and nitrogen (N) when cows were fed on diets containing 0 to 24% DDGS

	DDGS inclusion level					
	0	8	16	24	SED	P
Dry Matter	0.712	0.713	0.702	0.705	0.011	0.693
Nitrogen	0.708	0.706	0.678	0.673	0.012	0.016

Table 60 Digestibility of dry matter (DM) and nitrogen (N) for each combination of group and DDGS inclusion level

Group	Hi				Lo					
DDGS %	0	8	16	24	0	8	16	24	SED	P
Dry Matter	0.716	0.718	0.706	0.709	0.710	0.711	0.700	0.704	0.016	0.999
Nitrogen	0.709	0.712	0.691	0.675	0.707	0.702	0.672	0.673	0.018	0.903

5.5.5 Discussion

A lack of difference between treatments was anticipated because approximately 80% of dietary ingredients were identical. The results provide reassurance that inclusion of DDGS did not have an adverse effect on digestibility of dry matter. The lower digestibility of nitrogen at higher levels of DDGS inclusion was associated with lower dry matter intake and might have contributed to the negative relationship between milk yield and DDGS inclusion level.

To examine whether reduced nitrogen digestibility agrees with predictions, equations developed under our DairyCo Research Partnership (Garnsworthy and Wilkinson, 2012) were used to calculate expected faecal nitrogen output. These equations were derived from the Feed into Milk (FiM) MP system and use the elements that FiM equations discard. For example, FiM uses digestible undegraded protein (DUP) in the calculation of MP supply; the faecal model assumes that indigestible undegraded protein (iDUP) contributes to faecal nitrogen output. The model was run for the 0 and 24% DDGS inclusion levels, using observed values for DMI, lab values for nitrogen content of feeds, and NIR-predicted values for degradability characteristics of forages.

Observed nitrogen output was on average 26 g/d greater than predicted, so observed nitrogen digestibility was 4 percentage points lower than predicted for both diets (Table 61). An important point, however, is that nitrogen digestibility was predicted to be 4 percentage points lower for the diet with 24% DDGS inclusion, which agrees with the observed difference.

Table 61 Observed and predicted values for nitrogen (N) intake, output and digestibility at DDGS inclusion levels of 0 and 24%

	Observed		Predicted	
	0	24	0	24
N Intake (g/d)	664	637	666	633
N Output (g/d)	194	208	168	182
N digestibility	0.709	0.673	0.748	0.713

5.5.6 Conclusions

1. There was no significant effect of DDGS inclusion level on dry matter digestibility.
2. Nitrogen digestibility was lower for diets with higher DDGS inclusion levels, which is in agreement with retrospective calculations.
3. Book values suggest that differences in nitrogen digestibility might be attributed to lower digestibility of UDP for DDGS, as a result of heat treatment. Also, this batch of wDDGS had a lower proportion of solubles than batches produced later.

5.6 Respiration Chamber Trials

5.6.1 Objectives

To quantify the effect of wDDGS on methane emissions by dairy cows.

5.6.2 Background

Work at the Rowett Research Institute (analysed by Giger-Reverdin and Sauvant, 2000) showed that DDGS resulted in the lowest methane emissions of all concentrate feed ingredients when fed to sheep at maintenance; McGinn *et al.* (2009) reported a 20% decrease in methane emissions when DDGS replaced barley in diets for fattening beef cattle. In Dairy Trial 1, however, inclusion level of wDDGS-1 did not affect methane emissions by high-yielding dairy cows. The aim of the current study was to investigate methane emissions by dairy cows housed in respiration chambers when fed on diets containing 0 or 24% wDDGS-1.

5.6.3 Material and Methods

Six cows in mid-lactation were selected from the dairy herd and transferred from the research farm to respiration chambers for 5 days to measure daily methane output whilst still being fed on the

commercial diet. Cows were then transferred to standings and fed *ad libitum* on each of two levels of DDGS inclusion (0 and 24% from Dairy Trial 1) for 14 days before being transferred to respiration chambers for 5 days. Whilst in standings and respiration chambers, cows were milked and fed twice daily at 07:00 and 16:30. Methane output was recorded continuously whilst cows were in chambers and expressed as average 24-hour output and as output per kg DMI. Details of design and operation of respiration chambers are in Garnsworthy *et al.* (2012).

5.6.4 Results

When cows in respiration chambers were fed on the diet containing 24% wDDGS-1, they produced significantly less methane per day and per kg dry matter intake than when they were fed on the diet containing no DDGS or the commercial diet (Table 62). There was no effect of diet on dry matter intake or milk yield in respiration chambers. Dry matter intake and daily methane output were significantly lower when cows were housed in respiration chambers than when they were housed in the dairy centre and fed on the same diet.

Table 62 Methane output, dry matter intake and milk yield of six cows housed in respiration chambers and fed on a commercial diet or diets containing 0 and 24% wheat DDGS

	Diet in chamber							
	Commercial	0% wDDGS	24% wDDGS	SED[1]	P[2]	Farm[3]	SED[4]	P[5]
Methane (g/d)	353	360	298	11.2	0.002	396	10.3	0.008
Methane (g/kg DMI)	20.3	20.8	17.5	1.13	0.045	19.4	0.51	0.104
DMI (kg/d)	17.5	17.4	17.1	0.72	0.855	20.6	0.45	<0.001
Milk yield (kg/d)	21.7	21.1	20.6	1.89	0.839	25.3	2.39	0.189

[1] standard error of difference between diet means; [2] probability of difference between diets; [3] methane, intake and milk yield of cows at the dairy centre before transfer to respiration chambers; [4] standard error of difference between farm and chamber means; [5] probability of difference between farm and chamber.

5.6.5 Discussion

The results of this study concur with studies elsewhere which observed lower methane emissions when DDGS were fed to ruminants. In Dairy Trial 1, however, there was no effect of diet on daily methane emissions. Lower methane emissions might be expected when feeding DDGS due to less fermentable fibre and methane-inhibiting fatty acids in DDGS. The difference between trials in the current project is probably due to differences in level of feeding and milking system for cows at the Dairy Centre and in chambers.

At the Dairy Centre, individual cows were free to move around, to eat, and to present themselves for milking at any time of day or night; on average, cows were milked 2.9 times per day. In the standings and chambers, cows were restrained and were milked and fed twice-daily at fixed times. Consequently, although they were fed on the same commercial diet, milk yield was 4 kg/d lower (NS) and dry matter intake 3 kg/d lower (P<0.001) when cows were in the chambers than when they were at the Dairy Centre.

In the chambers, there was no difference in intake or milk yield between the commercial diet and either DDGS diet. This allows us to conclude that the lower methane output when cows were fed on the 24% DDGS diet was not due to reduced feed intake, which was a possible confounding factor in Dairy Trial 1.

5.6.6 Conclusions

This study supports the hypothesis that inclusion of wDDGS in dairy diets reduces methane output.

5.7 Dairy Production Trial 2

5.7.1 Objectives

To measure responses to wDDGS inclusion level in diets balanced for energy and nutrients.
To quantify the effect of wDDGS on methane emissions by dairy cows.

5.7.2 Background

In Dairy Production Trial 1, milk yield and dry matter intake were depressed by the highest level of wDDGS inclusion (24% of diet DM). Several factors could have contributed to this result:

- Overestimation of ME content for wDDGS in diet formulations;
- Incorrect rumen degradability values assumed for wDDGS protein in diet formulations;
- Insufficient starch or palmitic acid in diets containing 24% wDDGS.
-

The first two factors were explored in the Sheep and Rumen Trials, which confirmed that the batch of DDGS (wDDGS-1) used in Dairy production Trial 1 had lower ME and protein degradability values than had been assumed in diet formulations, which might have resulted in deficiencies of ME and ERDP. The Sheep and Rumen Trials also provided measured nutritive values for the batch of DDGS (wDDGS-3) available for Dairy Production Trial 2. These measured nutritive values could be used to improve accuracy of formulations, resulting in a better match of nutrient supplies across diets.

Our studies under a previous LINK project (LK0646) indicated that both starch and saturated fat are important for optimum performance and fertility in dairy cows. To eliminate these as possible

limiting factors, therefore, minimum amounts of wheat and saturated fat (palmitic acid) were included in formulations.

5.7.3 Material and Methods

Design

As for Dairy Production Trial 1, the trial design involved 4 levels of wDDGS inclusion in a Latin square. Following statistical analysis to confirm power, however, length of treatment period was reduced to 3 weeks, giving a total of 16 weeks. Each treatment period consisted of 2 weeks diet adaptation and 1 week recording. Levels of wDDGS inclusion were 0, 7.5, 15 and 22.5% on a DM basis. Replication was 41 cows in Periods 1 and 2, 38 cows in Period 3, and 36 cows in Period 4. A pre-trial period of two weeks was allowed, during which all cows were fed on the commercial diet. In the current trial, cows were not blocked according to stage of lactation because no interaction had been found in Dairy Production Trial 1; therefore, only one diet was formulated per wDDGS inclusion level.

Animal management, sampling and recording

These were the same as for Dairy Production Trial 1, except for the addition of rumen sampling during week 3 of each treatment period. Rumen fluid samples were collected by stomach tube for analysis of rumen pH and volatile fatty acid concentrations.

Diet Formulation

Diets were formulated with wDDGS included at 0, 7.5, 15 and 22.5% of total diet on a DM basis. Diets were designed to supply requirements for M+32 litres of milk production per day. Two diets were formulated to include 0 and 22.5% wDDGS – the middle two treatments were produced as composites of these two extremes. All treatment diets contained approximately 50% forage DM supplied by grass, maize and whole-crop silages in proportions 16:11:9. The concentrate portion of the Control PMR (0% wDDGS) contained wheat, beet pulp, soya, rape, fat, urea, and a premix containing minerals, vitamins and rumen conditioners; for the 22.5%-wDDGS PMR, most of the wheat and all of the beet pulp, soya, rape and urea were replaced by wDDGS and some SoyPass to ensure adequate bypass protein supply (Table 63).

Table 63 Formulations of partial mixed rations containing four levels of DDGS (values are kg fresh weight per cow per day)

DDGS %	0	7.5	15	22.5
Grass Silage	16.0	16.0	16.0	16.0
Maize Silage	11.0	11.0	11.0	11.0
Whole Crop silage	9.00	9.00	9.00	9.00
Chopped straw	0.50	0.50	0.50	0.50
DDGS	0.000	2.00	4.00	6.00
Sugar beet pulp	1.00	0.67	0.33	0.00
Soya bean meal	2.00	1.33	0.67	0.00
Rapeseed meal	2.75	1.83	0.92	0.00
Wheat-rolled	2.50	2.00	1.50	1.00
Fat supplement	0.25	0.25	0.25	0.25
SoyPass	0.00	0.27	0.53	0.80
Mineral & Vitamin mix	0.150	0.150	0.150	0.150
Sodium bicarbonate	0.100	0.100	0.100	0.100
Limestone Flour	0.125	0.125	0.125	0.125
Urea	0.080	0.053	0.027	0.000
Total:	45.5	45.3	45.1	44.9

Calculations and statistical analysis

These were the same as for Dairy Production Trial 1, except the main effect of Group (stage of lactation) was omitted from the statistical model.

5.7.4 Results

There was no effect of wDDGS inclusion level on feed intake, yields of milk, energy-corrected milk, fat, protein and lactose, or milk fat, protein or lactose concentrations (Table 64). Milk urea concentration was greater for 0 and 7.5% wDDGS compared with 15 and 22.5% wDDGS. There was no effect of wDDGS inclusion level on live weight, live-weight gain or body condition score. There was no effect of wDDGS inclusion level on feed conversion efficiency or nitrogen use efficiency.

Table 64 Mean intake, performance and efficiency when cows were fed on diets containing 0 to 22.5% wDDGS-3

	wDDGS-3 inclusion level, %					
	0	7.5	15	22.5	SED	P
DMI, kg/d	22.9	22.5	22.2	22.1	0.36	0.188
PMR DMI, kg/d	18.1	17.8	17.5	17.7	0.35	0.383
conc DMI, kg/d	4.8	4.7	4.7	4.4	0.21	0.240
ME Intake, MJ/d	277	274	271	270	4.4	0.354
Milk yield, kg/d	32.6	32.4	31.8	31.6	0.64	0.347
ECM yield, kg/d	32.3	32.8	32.1	31.7	0.69	0.473
Fat, kg/d	1.25	1.29	1.26	1.24	0.037	0.584
Protein, kg/d	1.14	1.13	1.11	1.10	0.022	0.199
Lactose, kg/d	1.45	1.47	1.45	1.43	0.037	0.799
Fat, %	3.91	4.08	4.08	4.01	0.088	0.188
Protein, %	3.51	3.55	3.51	3.50	0.035	0.579
Urea, mg/dl	38.3	37.0	34.1	33.5	1.04	**<0.001**
Lactose, %	4.51	4.51	4.54	4.54	0.016	0.106
Live weight, kg	711	709	704	706	1.5	0.107
LWG, kg/d	0.29	0.44	0.22	0.23	0.09	0.095
BCS	3.23	3.20	3.22	3.18	0.01	0.702
FCE	0.17	0.17	0.17	0.17	0.004	0.677
NUE	0.30	0.30	0.29	0.29	0.007	0.209
NUE-TP	0.27	0.28	0.26	0.27	0.009	0.394

SED, standard error of difference for comparing group means; P, F-ratio probability; DMI, dry matter Intake; PMR, partial-mixed ration; conc, robot concentrate; ME, metabolisable energy; ECM, energy-corrected milk; LWG, live-weight gain; BCS, body condition score; FCE, feed conversion efficiency; NUE, nitrogen use efficiency; NUE-TP, nitrogen use efficiency for true protein synthesis.

There was no effect of DDGS inclusion level on plasma protein fractions, urea-N, β-hydroxybutyrate, NEFA or glucose (Table 64).

Table 65 Plasma indicators of protein and energy status when cows were fed on diets containing 0 to 22.5% DDGS

	wDDGS inclusion level, %					
	0	7.5	15	22.5	SED	P
Albumin, g/l	34.3	33.7	33.6	34.6	0.76	0.567
Globulin, g/l	39.9	40.4	41.2	41.0	1.33	0.733
Total Protein, g/l	74.2	74.1	74.8	75.6	0.98	0.412
Urea-N, mmol/l	5.5	5.4	5.3	5.2	0.15	0.170
BOHB, mmol/l	0.55	0.55	0.52	0.52	0.031	0.725
NEFA, mmol/l	0.08	0.10	0.09	0.10	0.011	0.260
Glucose, mmol/l	3.68	3.66	3.67	3.70	0.045	0.361

SED, standard error of difference for comparing treatment means; P, F-ratio probability; BOHB, β-hydroxybutyrate; NEFA, non-esterified fatty acids

There was no effect of DDGS inclusion level on daily methane output, methane per kg DMI, time spent ruminating, time between boluses or time between chews (Table 66). There was no effect of DDGS inclusion level on rumen pH, acetate, propionate, butyrate, isovalerate and total VFA, but isobutyrate concentration and molar percentage decreased with inclusion level and valerate percentage increased with inclusion level.

Table 66 Methane output and rumen parameters when cows were fed on diets containing 0 to 22.5% wDDGS

	wDDGS inclusion level, %					
	0	7.5	15	22.5	SED	P
Methane, g/d	432	436	423	434	7.08	0.310
Methane, g/kg DMI	19.4	19.9	19.7	19.8	0.59	0.823
Rumination, min/d	443	432	428	427	8.0	0.227
Bolus interval, s	54.9	54.7	55.4	55.0	0.25	0.065
Chew interval, ms	84.4	85.2	83.7	86.8	1.46	0.180
Rumen pH	6.72	6.68	6.71	6.73	0.053	0.698
Acetic, mmol/l	72.9	71.5	70.9	66.7	3.19	0.260
Propionic, mmol/l	29.5	29.6	29.4	27.8	1.74	0.725
Butyric, mmol/l	17.6	17.6	17.9	17.1	1.05	0.914
Isobutyric, mmol/l	1.42	1.40	1.31	1.23	0.06	**0.011**
Isovaleric, mmol/l	1.83	1.78	1.72	1.59	0.10	0.126
Valeric, mmol/l	1.91	1.93	2.06	1.98	0.14	0.717
Total VFA, mmol/l	125	124	123	117	6.0	0.496
Acetic, molar%	58.6	58.2	57.7	57.3	0.53	0.076

Propionic, molar%	23.3	23.6	23.9	23.9	0.47	0.501
Butyric, molar%	13.9	14.1	14.3	14.7	0.32	0.142
Isobutyric, molar%	1.2	1.2	1.1	1.1	0.04	**0.032**
Isovaleric, molar%	1.5	1.4	1.4	1.4	0.05	0.201
Valeric, molar%	1.5	1.5	1.6	1.7	0.06	**0.012**

SED, standard error of difference for comparing treatment means; P, F-ratio probability

5.7.5 Discussion

The results of this trial confirm that diets for dairy cows can be formulated to include up to 22% wDDGS (on a DM basis) without any significant impact on dry matter intake or performance. Although average performance in the current trial (ECM 32 l/d) was lower than in Dairy Production Trial 1 (ECM 40.0 l/d), the range of energy-corrected milk yield (14 to 52 l/d) was similar to the range observed in Trial 1 (23 to 66 l/d). The difference in milk yield between trials was due to a 2-month delay in starting the current trial. Importantly, there was no interaction between DDGS inclusion level and either stage of lactation or milk yield in either trial. This means that diets containing up to 22.5% wDDGS can support milk yields across the complete range encountered in normal commercial practice, provided diets are balanced for all major nutrients.

Intake and milk yield were not affected by DDGS inclusion level in the current trial, whereas in Trial 1 both were depressed when cows were fed on a diet containing 24% DDGS. Eliminating the depression cannot be ascribed to a single factor because several factors were changed between trials. We can deduce, however, that there is nothing intrinsic to DDGS that caused the depressions in Trial 1; the most likely explanation is that the diet containing 24% DDGS was deficient in supply of ME or a key nutrient. Supplementary sources of starch and saturated fat appear to be beneficial for maintaining dry matter intake and milk yield with diets containing high proportions of DDGS.

5.7.6 Conclusions

The main conclusions from this study are:

1. Level of wDDGS inclusion did not influence dry matter intake, milk yield or major milk constituents, but higher wDDGS levels reduced milk urea concentration.
2. Level of wDDGS inclusion did not influence methane emissions, which were generally low.
3. Results of this study are in contrast to Dairy Production Trial 1, where dry matter intake and milk yield were depressed by the highest level of wDDGS inclusion; this is thought to be due to basing formulations on determined ME and degradability values, and providing supplementary starch and saturated fat across all diets.
4. Results of this study provide further support for the conclusion that wDDGS can be included in diets for dairy cows at up to 20% without detrimental effect on cow performance or health.

5.8 Commercial Beef Study

5.8.1 Objectives

To gather opinions of beef farmers who had used wheat DDGS in growing and finishing systems.

5.8.2 Background

There is an established market for feeding DDGS to beef cattle, based mainly on maize DDGS from USA or wheat DDGS and barley DDGS from UK whisky distilleries. Because the ENBBIO programme has provided improved knowledge about the nutritive value of wDDGS from UK bioethanol production, more precise ration formulation should be possible. Whether the benefits of this approach translate into practice on commercial farms needed to be tested.

The original intention was to conduct simple A v B or five treatment beef trials with wDDGS on two or three commercial beef farms. Despite exhaustive efforts, willing farmers with suitable farms could not be found. Commercial farms simply do not have adequate penning, weighing facilities and ability to feed discrete diets, and farmers were not keen on the extra work involved given the low beef price at the time. Attention moved to college farms, but costs of running trials were prohibitive. The Consortium agreed, therefore, to carry out case studies with commercial farmers using wDDGS in growing and finishing cattle, basically getting their views on how wDDGS compares to other proteins they have used and any practical issues they have with the product.

5.8.3 Material and Methods

A questionnaire was designed to gather and record information and opinions from beef farmers that had used wDDGS. The questionnaire was completed by an independent researcher during face to face interviews on farms. Due to time constraints, only two interviews could be conducted.

5.8.4 Results

Farm A	
Beef enterprise details:	200-250 finishers, 30 suckler cows Finishers: Friesian bullocks, bought in
Use of DDGS:	Started using DDGS more than 18 months ago because of price. DDGS replaced maize gluten and protein premixes. DDGS is fed to finishing cattle along with a cereal blend (barley and wheat). DDGS is roughly 30% of the diet. Some DDGS is fed neat with silage depending on the size and condition of the stock. The type of DDGS depends on supply, at the moment

	it is wheat, previously used maize and European DDGS. Some TraffordGold is used in the final stages of finishing for starch.
Cattle performance:	Cattle start at 6-8 months and are finished at 18/20 months with carcase weight 320-340 kg. Deadweight gain is approximately 0.5-0.6 kg/day. Fat class O- and O+.
Buying DDGS:	DDGS is cheaper than other feeds Purchased for both protein and energy content Buys as required rather than buying forward
Comments on using DDGS:	Good points: DDGS is cheap, with good protein and energy content. Bad points: None. Intentions: will continue using DDGS next year as happy with performance.

Farm B	
Beef enterprise details:	Limousin cross sucklers (autumn calving) 200 homebred Beef Cattle finished annually.
Use of DDGS:	Started using DDGS in 2011, to reduce feed cost. DDGS replaced soya DDGS fed to Bulls and Heifers at 12.5% of diet Diet is Barley (69%), DDGS (12.5%), Sugarbeet (7.5%), Molasses (7%), Minerals, Yeast Uses wheat DDGS in meal form, but prefers pellets
Cattle performance:	Cattle are finished from 2 months to 18 months of age. Sold through live market Bulls weigh 740-780kg LWT at sale.
Buying DDGS:	DDGS is more competitive than soya on a protein basis Purchased for both protein and energy content Buys forward and as required.
Comments on using DDGS:	Good points: DDGS is easy to feed. Bad points: Meal sometimes left in the bottom of the hoppers when fed to sheep. Intentions: will continue using DDGS next year.

5.8.5 Conclusions

Both farmers are getting good performance from cattle fed on wheat DDGS.
Both purchase DDGS as a cheaper source of protein and energy than other protein feeds.
Both intend to continue using DDGS next year and had no practical issues with the product.

5.9 General Discussion and Summary of Ruminant Studies

The ENBBIO ruminant studies achieved the primary objective, which was to evaluate Wheat DDGS (wDDGS) from UK bioethanol production in terms of nutritional value and animal responses to inclusion in typical ruminant diets.

The first dairy trial was designed to find the limitations of wDDGS inclusion, which appeared to be approximately 20% of diet dry matter when diets were formulated to be balanced for energy and nutrients using existing knowledge and assumptions about the nutritive value of wDDGS. At the highest inclusion level (24% of diet DM), dry matter intake and milk yield were lower than for the control diet, although average milk yield for this treatment exceeded 41 litres per day. Various hypotheses were proposed to explain the lower performance with the highest level of inclusion:

- Overestimation of ME content of wDDGS
- Incorrect values for degradability characteristics of wDDGS, leading to shortage of protein
- Altered rumen fermentation
- Reduced digestibility of whole diet or protein fraction at high levels of wDDGS inclusion
- Imbalance or shortage of key nutrients, such as starch or saturated fatty acids

These hypotheses were tested in a series of feed evaluation studies.

Results of sheep ME trials provided evidence to support the hypothesis that the wDDGS used in the first dairy trial had a lower ME content than was assumed during diet formulation. The ME value used for diet formulation was 13.7 MJ/kg DM and the value measured in the sheep ME trial was 12.1 MJ/kg DM. At 24% inclusion level, actual ME intake was 9 MJ/d lower than formulations. This level of ME reduction is equivalent to ME requirements for nearly two litres of milk per day, which was the difference observed between 0% and 24% wDDGS in the dairy trial.

Results of rumen studies showed that degradability characteristics of DDGS vary markedly between sources, probably as a result of proportion of solubles added and heat treatment during drying. Degradability characteristics of DDGS have a profound effect on calculated supplies of ERDP and MP, which can make the difference between deficiency and surplus in diet formulation. A possible shortage of ERDP in the first dairy trial was identified, which would have been consistent with reduced feed intake.

Rumen fermentation studies did not support the hypothesis that rumen pH or VFA proportions were influenced sufficiently to explain performance responses observed in the first dairy trial.

Digestibility studies confirmed that there was no significant effect of wDDGS inclusion level on dry matter digestibility. Nitrogen digestibility was lower for diets with higher DDGS inclusion levels, however, which is in agreement with retrospective calculations. Given the calculated surplus of MP, it is unlikely that reduced nitrogen digestibility can explain treatment differences observed in the first dairy trial.

A second dairy trial was designed to re-examine the effect of inclusion level of wDDGS. For this trial, diets were formulated with ME values and degradation characteristics determined in advance for the actual batch of wDDGS to be tested. Furthermore, developments in the DDGS production process since the first dairy trial, particularly increased proportion of syrup, had improved the ME concentration and degradability characteristics of the wDDGS available. Levels of wDDGS inclusion ranged from 0 to 22.5% of diet DM. There was no effect of wDDGS inclusion level on intake or performance.

In a questionnaire based on wDDGS use on commercial beef farms, inclusion levels of 12.5% and 30% of the diet was reported by the farmers to support good performance levels.

Important considerations during formulation of diets containing high proportions of DDGS are:

- Accurate estimations of ME value and degradability characteristics are required.
- ME value and degradability characteristics vary with type of DDGS.
- Ensuring adequate supplies of starch and saturated fat appears to be beneficial for maintaining dry matter intake and performance.

6 Enhancing the nutritional value of DDGS through processing

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Abstract:

Overview of the activities and achievements of the Processing Subgroup

The remit of the Processing Subgroup of ENBBIO was to investigate options to improve the nutritional value of DDGS through processing. Four main options were identified and investigated:

1. Extraction of Arabinoxylans (AX) from DDGS. AX are non-starch polysaccharides that increase viscosity and reduce nutrient availability in animal feed formulations. Removing the AX from DDGS would reduce its soluble fibre content and make it more suitable for non-ruminant animal feed; meanwhile AX is potentially a valuable product in its own right that could provide an additional revenue stream for a wheat biorefinery. AX was quantified in DDGS and in fractions produced by dry fractionation, and extractions performed. While the principle was highlighted that removal of AX from DDGS is a potential means for enhancing both the nutritional value of DDGS and the economics of a wheat biorefinery, the yields in the current work were low. Nevertheless, the current work added to the argument that AX extraction is an option with real potential for altering the nature and use of co-products from ethanol biorefining, including improving the nutritional value of DDGS.

2. Dry fractionation of DDGS by sieving and elutriation. The particles of DDGS vary in size, shape and composition, and separation based on size and shape could yield fractions varying in composition and therefore better suited to end-use applications or to further processing. Sieving separates into fraction based on size, while elutriation (air aspiration) separates based on aerodynamic properties that include size, shape and density. Combinations of sieving and elutriation have been successfully applied elsewhere to Maize DDGS. The effects of dry fractionation of Wheat DDGS using sieving, elutriation and combinations of the two were investigated in the current work. A picture emerged in which large/heavy particles appeared to be agglomerates of several types of material held together with syrup, while small/light particles tended to be more distinct in their botanical origin. Unfractionated DDGS had a crude protein content of 32.7% and a crude fibre content of 8% (on a 10% moisture basis). Fractionation by sieving gave a coarse fraction enriched in protein to 33.9% and depleted in crude fibre to 7.2%,

and a fine fraction with protein and fibre contents of 31.9% and 9.3%, respectively. Elutriation was able to give greater levels of differentiation to produce a heavy fraction enriched in protein to 34.9% and depleted in crude fibre to 6.4%, and a light fraction with protein and fibre contents of 31.4% and 9.4%. These changes were too small to be of commercial interest, with the effects of sieving or elutriation limited by the agglomerated nature of the DDGS particles; applying these approaches to DDG without S (*i.e.* particles that had not been "glued" together into agglomerates by the syrup material) is likely to give better differentiation. The results demonstrate that this sort of fractionation has potential for application within a more sophisticated biorefinery, in order to produce streams more tailored to specific end-uses or further processing; for example, a fibre-enriched stream would be a more promising source from which to extract arabinoxylans.

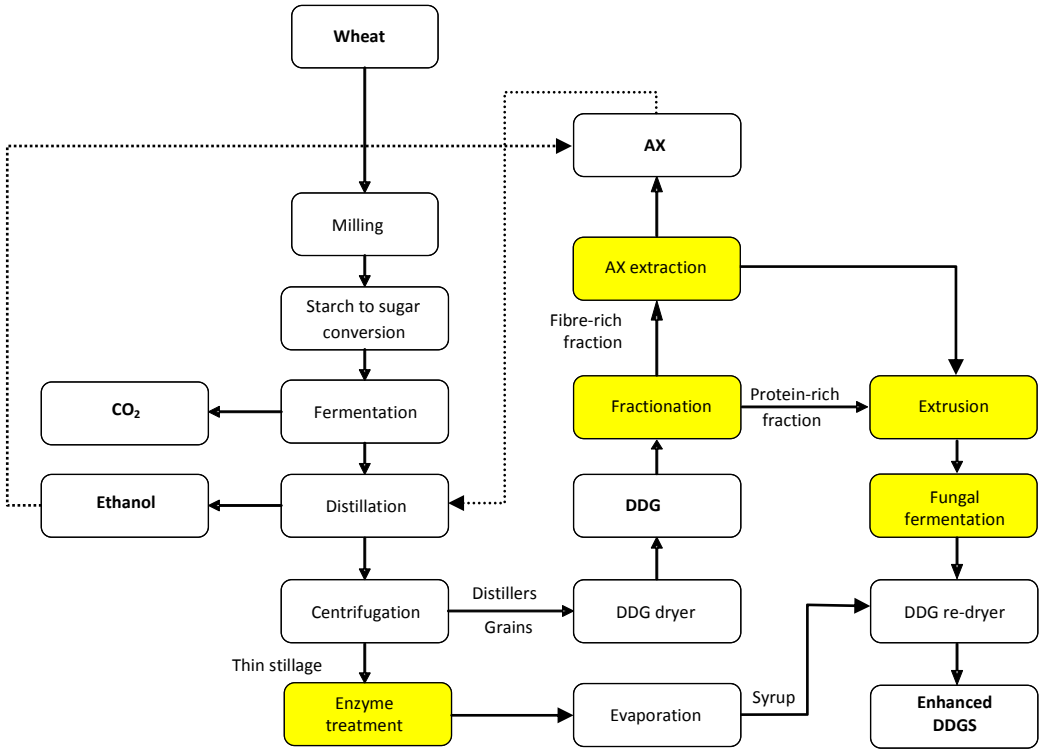
3. Fungal fermentation of DDGS to alter its amino acid composition. The value of DDGS for animal feed is limited by its high fibre content and by its relatively poor protein quality. The above two approaches for improving the nutritional value of DDGS focused on reducing the fibre content by removing AX (which would at the same time increase protein content) or by separating fibre- and protein-rich material. This third activity focused instead on improving the protein quality by altering the amino acid composition. Solid state fermentation of DDGS was carried out using the fungi *Aspergillus oryzae* and *Aspergillus awamori*, in order to turn DDGS protein into fungal protein with, it was hoped, a different amino acid composition. Profiling of 18 amino acids was carried out on the original DDGS and on DDGS fermented with the two strains individually and with mixed cultures. Principal component analysis demonstrated that fermentation altered the amino acid composition favourably towards greater levels of lysine and alanine and decreased glutamic acid and proline. The improvement appeared to be greater for mixed-culture samples than for mono-culture samples. The ratio of essential:total amino acids was also improved, indicating that fungal fermentation offers a basis for improving the nutritional quality of DDGS in terms of its amino acid composition. This work was carried out via an HGCA-funded undergraduate studentship; the main findings are summarised briefly in the current report.

4. Intense mechanical working of DDGS to alter protein structure. Instead of altering the DDGS protein composition, the possibility of altering the protein structure via intense mechanical mixing was investigated. The hope was to enhance levels of Rumen Bypass Protein. Samples of DDGS were extruded in a twin-screw extruder at water contents of 30 and 38%, temperatures of 25 and 60°C, and screw speeds of 300 and 500 rpm. Extruded samples were tested for degradability using Dacron bag studies performed at the University of Nottingham. All treatments greatly increased Dry Matter and Nitrogen degradability, slightly more so at the lower temperature. DM degradability for the DDGS was 0.53, increasing on average to 0.82 after extrusion at 25°C and 0.79 after extrusion at 60°C; similarly, N degradability for the DDGS was 0.39, increasing on

average to 0.85 after extrusion at 25°C and 0.81 after extrusion at 60°C. Thus intense mechanical work input has the potential to significantly alter DDGS digestion.

In addition to the above investigations of options to improve the nutritional value of DDGS through processing, a study was performed on ***the potential for enzymatic reduction of syrup viscosity***. Syrup obtained from the Ensus plant was incubated with a range of enzymes (xylanase, two proteases, beta-glucanase, alpha-amylase, a beta-glucanase/xylanase mix, and a mix of saccharifying enzymes). Viscosity was measured using a Rapid-Visco Analyser. Results were variable, as a result of varying feedstock and operating conditions at the Ensus plant giving varying syrups; generally the enzymes gave some viscosity reduction, with the greatest reductions from the beta-glucanase/xylanase mix. In general it was demonstrated that despite the use of enzymes within the bioethanol process, there remained significant scope to reduce syrup viscosity further, which would allow syrup to be produced at greater concentrations, reducing transport costs.

The figure below illustrates how these five innovations could be deployed to extend a conventional bioethanol plant to produce an additional arabinoxylan co-product and an enhanced DDGS with superior nutritional performance for animal feed, whilst providing enhanced scope for process integration.



An illustrative concept of an integrated biorefinery that extends the conventional bioethanol production process by employing the innovations explored in this report (in yellow): dry fractionation, AX extraction, extrusion, fungal fermentation and enzyme treatment of the syrup, to produce an Enhanced DDGS and an additional Arabinoxylan co-product.

6.1 Introduction

The emergence of wheat bioethanol plants in the UK in recent years has increased the availability of Wheat DDGS for use in animal feed formulations. The main focus of the ENBBIO project was to evaluate the nutritional performance of this DDGS in animal feed. The focus of this part of the project was on indentifying ways to improve the nutritional value of DDGS through processing.

The Processing Sub-group comprised: Dr Grant Campbell, University of Manchester; Dr Hosam Aleem, University of Manchester; Dr James Brosnan, SWRI; Dr Reg Agu, SWRI; Dr Harley Stoddart, AHDB-HGCA; Dr Richard Weightman, ADAS; Dr Michael Marsden, AB Agri Ltd.; John Pinkney and Sam Cotterill, Ensus plc. The remit of the Processing Sub-group was defined in Tasks 3.1 and 3.3 of the project plan: *Assess potential modifications to production processes in the biorefinery*, and *Explore potential to modify viscosity and availability of nutrients through chemical/physical approaches*. Material for the studies was provided by the Ensus bioethanol plant. Due to the sensitivity of the Ensus process to any changes in operating conditions, it was decided in the first meeting of the Processing sub-group in May 2012 that the direct focus of the work should be on modification of the DDGS as currently produced in its final form, rather than by modifying the current process, but with the aim of recommending process modifications that could be introduced into bioethanol plants or wheat biorefineries following full contextual techno-economic evaluation.

There are two broad approaches by which the nutritional value of DDGS could be enhanced:

(i) Fractionate the DDGS into more differentiated streams with enhanced nutritional value or enhanced value for alternative processing or end-use;
(ii) Alter the chemical composition or physical structure of the DDGS through further processing.

Options were considered within the framework of an integrated biorefinery concept, in which value is added through fractionation and conversion processes that produce a range of revenue streams, whilst giving scope for process integration to enhance efficiency and hence economics (Campbell *et al.* 2006). Within this overarching framework, four options were identified as promising avenues for enhancing the nutritional performance of DDGS within the context of an integrated biorefinery:

1. Extraction of arabinoxylans
2. Dry fractionation of DDGS by sieving and elutriation
3. Fungal fermentation of DDGS to alter its amino acid composition
4. Intense mechanical working of DDGS to alter protein structure

The first two of these options are examples of the fractionation approach, in which the merit of the option depends not only on the resulting streams but on the context in which they are produced.

Thus, the process for extracting arabinoxylan employs ethanol; the feasibility of the AX option thus arises from the context of the bioethanol plant. Similarly, the dry fractionation approach may produce enriched fibre fractions that would be more suited to arabinoxylan extraction, illustrating again the integrated thinking that is required to conceive and evaluate possible processing options. Meanwhile, the latter two options are, respectively, examples of altering the chemical composition or physical structure through further processing.

In addition to these four studies, the potential for enzymatic reduction of syrup viscosity was investigated, as high viscosity limits the concentration of syrup and increases transportation costs. Section 7.3 describes the approaches taken for each of these five investigations, and Section 7.4 presents the results. Prior to this, to provide context for the work and its interpretation, the bioethanol process from which DDGS arises as a co-product is described.

In addition to the members of the Processing Sub-group listed above, the authors would like to acknowledge gratefully the contributions of several people to the studies presented here:
Akanksha Chawla and Rana Hassan Naji for MEng and MSc student projects at the University of Manchester on fungal fermentation and on AX contents of samples from dry fractionation, respectively; Ruth Bell at the University of Manchester and Dr Nikolina Cukelj visiting from Zagreb University for help with arabinoxylan studies; Mike Robinson of Sciantec for numerous analyses; Dr Nell Masey O'Neill of AB Vista and Dr Lorraine Salmon of Premier Nutrition for helpful comments on the fungal fermentation studies; Dr Andrew Plunkett at Manchester Metropolitan University for access to the extruder used in the mechanical work studies; Prof. Phil Garnsworthy at the University of Nottingham for Dacron bag studies of DDGS digestibility following extrusion.

6.2 The bioethanol production process

Figure 25 shows the bioethanol process operated by Ensus (www.ensusgroup.com), one of the industrial partners on the ENBBIO project. This is a typical bioethanol production process with the numbers in Figure 7.1 referring to the different process stages: (1) Grain Handling, (2) Mixing, (3) Mashing, (4) Cooking, (5) Liquefaction, (6) Cooling, (7) Fermentation, (8) Distillation, (9) Dehydration, (10) Storage and (11) Stillage. The current study focuses on the Stillage stage of the process that comprises the slurry from the bottom of the ethanol distillation column, also known as spent wash or thin stillage. The spent wash goes through a centrifugation process that separates the coarse grain solids from the solubles. The resulting stream rich in particulate solids is known as the Wetcake. The centrate stream with the solubles is then concentrated by evaporation into a syrup. The wetcake and syrup are then dried together in a rotary dryer to give Distillers' Dried Grains with Solubles (DDGS). Alternatively, the syrup may be sold separately to livestock producers, transported in tankers in which the concentration of syrup is limited by its viscosity. A focus of the current work was therefore to understand origins of syrup viscosity in order to identify

how viscosity might be reduced, hence allowing higher concentrations of syrup and lower transportation costs.

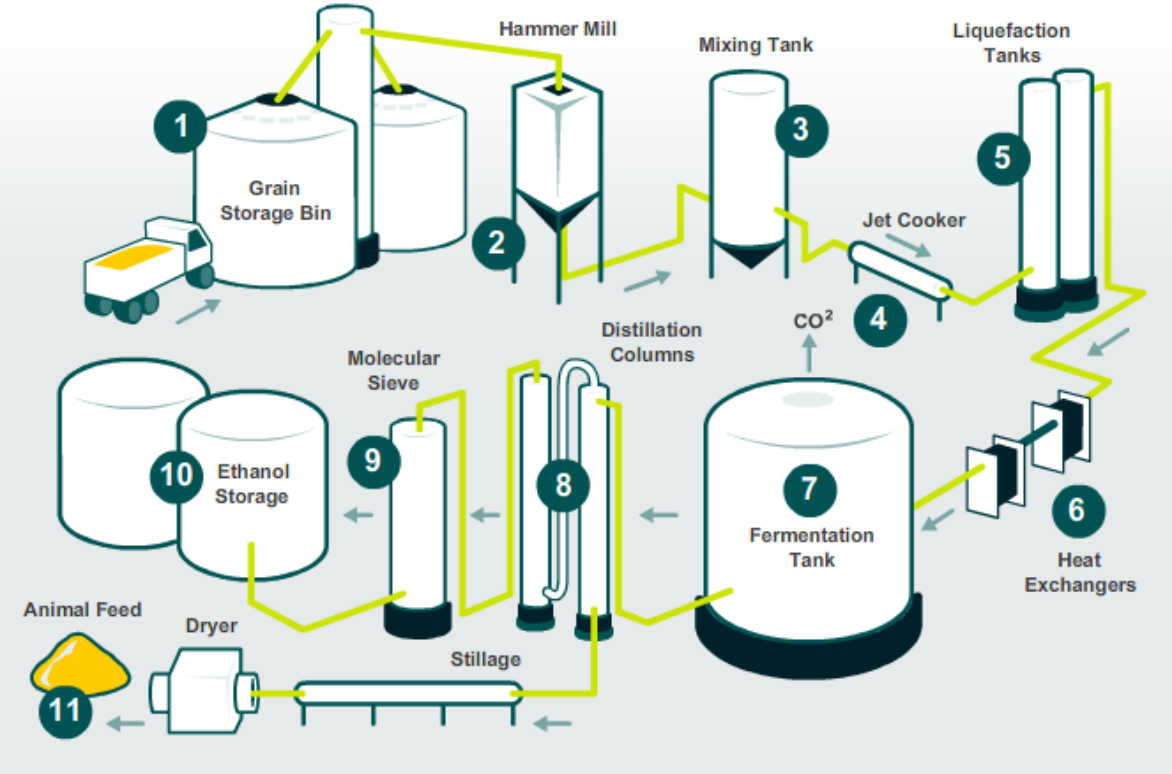


Figure 25 The Ensus Bioethanol production process, from the Ensus website www.ensusgroup.com.

6.3 Materials and Methods

This section describes the experimental studies on arabinoxylan extraction from DDGS, dry fractionation of DDGS, fungal fermentation of DDGS and intense mechanical working of DDGS, all with the aim of indentifying ways by which the nutritional value of DDGS for animal feed might be enhanced. DDGS for these studies was obtained from the stockpile of material supplied by Ensus for the ENBBIO project as a whole. The section also describes the studies of enzymatic reduction of syrup viscosity.

6.3.1 Extraction of Arabinoxylans from DDGS

The arabinoxylan content of DDGS and of fractions or extracts was measured by direct acid hydrolysis based on the procedure of Hollmann and Lindhauer (2005) and Du *et al.* (2009). Samples were hydrolysed with 1M sulphuric acid at 121°C for 1 hour, then neutralised to pH 7 with 1M sodium hydroxide. Samples were analysed by HPLC as described in Du *et al.* (2009). The AX content was calculated as 0.88×(Arabinose + Xylose), with the concentrations of arabinose and xylose calculated from HPLC traces using calibration curves.

Arabinoxylans in wheat comprise water-extractable arabinoxylans (WE-AX) and water-unextractable arabinoxylans (WU-AX) (Courtin and Delcour, 2002). The latter can be accessed using alkaline or enzyme extraction to produce alkaline-extractable arabinoxylans (AE-AX) and enzyme-extractable arabinoxylans (EE-AX), respectively. Figure 26 summarises the process for extracting WE-AX. In the current work yields were low and time constraints prevented extensive studies to improve extraction; this work is ongoing via subsequent projects, and is not reported in detail here. However, the above procedure for measuring AX contents was applied to understand the potential for dry fractionation of DDGS, which is described in the next section.

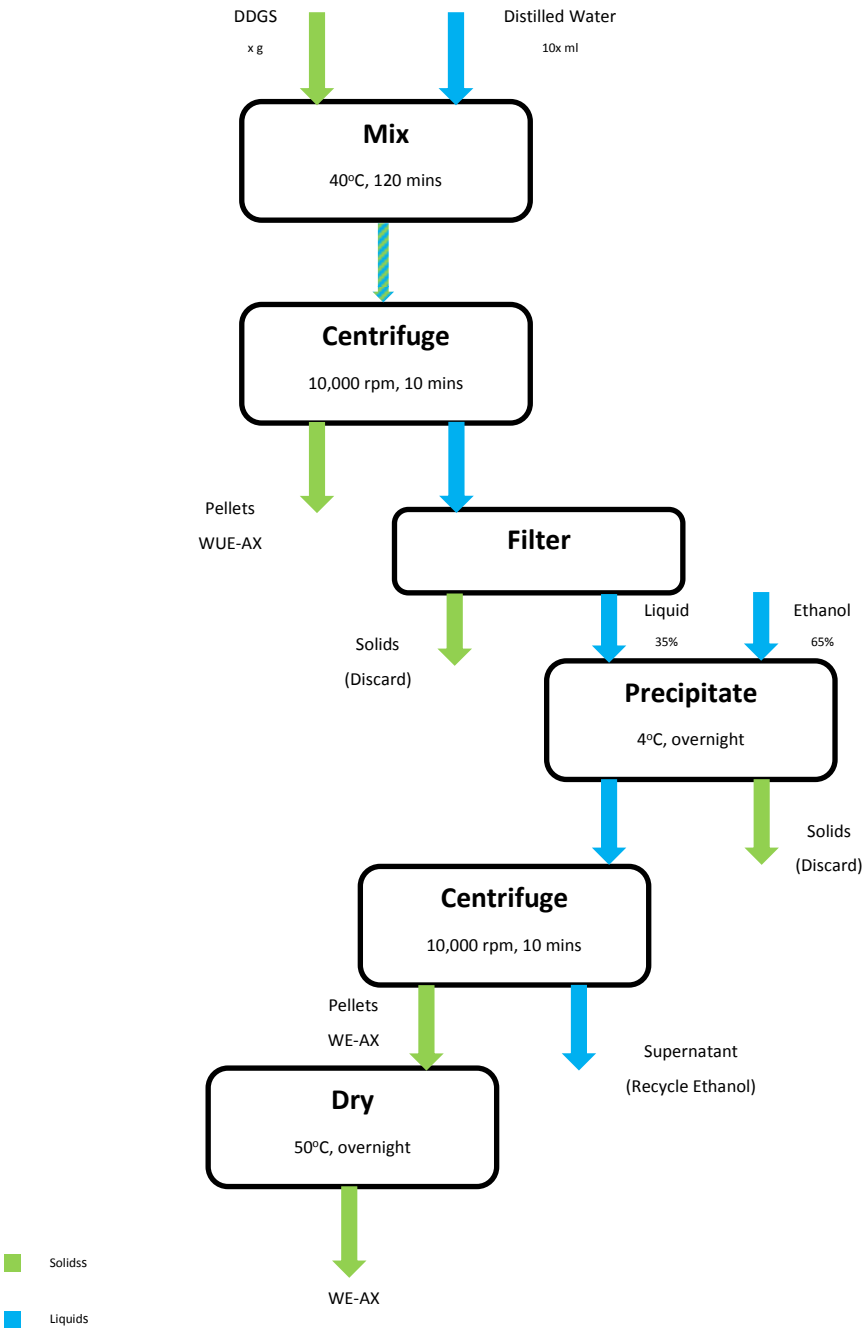


Figure 26 Process for extracting Water Extractable Arabinoxylan from DDGS.

6.3.2 Dry fractionation of DDGS by sieving and elutriation

The Wheat DDGS produced from the Ensus plant is a particulate material containing particles varying in size and shape; these particles probably also vary in composition and hence in nutritional value. Previous work on Maize DDGS has shown that the particles can be separated by sieving and elutriation to give fractions enriched or depleted in fibre or protein; this has been developed into a process called the Elusieve process (Srinivasan *et al.*, 2008, 2009; Pandya and Srinivasan, 2012; Pandya *et al.*, 2013). The current work aimed to investigate the potential of this approach for Wheat DDGS.

DDGS was separated into fractions based on size using sieving, and based on size, shape and density by elutriation (aspiration with air). Initially DDGS was sieved into separate fractions using a Satake Laboratory Plansifter (Satake Corporation, Hiroshima, Japan) with sieves of 2000 and 850 μm, along with a bottom pan, to give three fractions, Fine, Medium and Coarse. Five batches each of 200 g were separated. DDGS was also separated by elutriation using the Satake Paddy Rice Dehusker (Satake Corporation, Hiroshima, Japan) shown in Figure 27. The dehusker uses an upward flow of air to lift material from a flowing stream; heavy particles fall directly into a collection tray to the lower right of the figure, very light particles are lifted upwards and separated from the air stream using the cyclone at the left of the figure, and intermediate particles fall into a second collection tray. Thus the dehusker allowed production of three fractions: Light, Medium and Heavy. Because elutriation depends partly on particle size, these three fractions correspond loosely to the Fine, Medium and Coarse fractions obtained from sieving, and some later graphs will show the fractions from the two processes grouped together in this way. The dehusker has a vent that allows some control of the air flow; different levels of separation were achieved by operating the dehusker under Low flow and High flow (*i.e.* with the vent completely open or closed). Again, five batches each of 200 g were separated at each flowrate.

Figure 27 Satake paddy rice de-husker

Combinations of sieving and elutriation, and multiple elutriations, were also investigated, to refine the fractions in the hope of obtaining more effective compositional separation. Three variations were explored: sieving followed by elutriation, elutriation followed by sieving, and multiple elutriations. The logic was that, because of the difference bases for separation, particles falling into the same size fraction following sieving may well have differing shape and densities and hence differing responses to elutriation; elutriation could therefore be applied to these fractions to give more narrowly defined fractions. Equally, the light fraction obtained by elutriation, for example, could contain a combination of small, spherical, high density particles and large, flat, low density particles, the differences reflecting different botanical origins and different compositions; separating these by size, it was hypothesised, could give better differentiated fractions with greater compositional distinction. Meanwhile, although sieving is an absolute separation mechanism (if continued to completion) – particles in different fractions are absolutely larger or smaller – elutriation is less absolute in that the elutriation process is not completely efficient in its separation. Therefore, further elutriation of, say, the Light fraction could separate off some Very Light material that is even more compositionally distinct; similarly, further elutriation of the Heavy fraction could remove some lighter material to leave the remaining material more pure.

Sieving followed by elutriation

DDGS was separated into three fractions by sieving five batches each of 200 g, using 2000 and 850 μm sieves as described above. Each size fraction was then elutriated separately in the dehusker operating at a High air flowrate for all three fractions, and at a Low air flowrate for the Fine fraction.

Elutriation followed by sieving

DDGS was separated into three fractions by elutriating five 200 g batches in the dehusker at the High air flowrate. 100 g of each fraction was then separated by sieving using the Satake Laboratory Sifter and sieves of 2000, 1400, 850, 500 and 212 μm, along with a bottom collecting pan. Based on the results of this more detailed particle size analysis of each elutriated fraction, each fraction was then divided into two fractions of almost equal weight; the Light fraction was separated into two fractions using a sieve of 850 μm, the Medium fraction using a sieve of 2000 μm and the Heavy fraction using a sieve of 3150 μm.

Multiple elutriations

DDGS was separated into three fractions by elutriation in the dehusker at the High air flowrate. Each fraction was then elutriated again at the High flowrate. This resulted in seven new fractions: the Light fraction produced Light and Medium particles on further elutriation, but (practically) no Heavy particles; the Heavy fraction produced Heavy and Medium particles, but no Light particles, and the Medium fraction yielded Light, Medium and Heavy particles.

Table 69 in Section 6.4.3 summarises the investigations and the fractions produced. The fractions produced from all of the above studies were analysed by one of the project partners, Sciantec Analytical Services Ltd., for Crude Protein, Crude Fibre, Neutral Detergent Fibre, Oil B and Ash. The results of this investigation are presented in Section 6.4.3.

Arabinoxylan contents of sieved and elutriated fractions

In a separate study carried out as part of an MSc project carried our by Rana Hassan Naji, DDGS was fractionated by sieving and by elutriation as described above, and the arabinoxylan contents of the fractions measured as described in Section 6.3.1.

6.3.3 Fungal fermentation of DDGS to alter its amino acid composition

Solid state fermentation of DDGS was carried out using the fungi *Aspergillus oryzae* (a prolific protease producer) and *Aspergillus awamori* (a prolific amylase producer), in order to turn DDGS protein into fungal protein with, it was hoped, an amino acid composition that was nutritionally better for animal feed. This work was carried out by Akanksha Chawla via an HGCA-funded undergraduate studentship, the report of which is available separately (Chawla *et al.*, 2013). Briefly, DDGS was fermented with the two strains individually and with mixed cultures. The fermented samples were analysed for their amino acid profiles by Sciantec Analytical Services Ltd. using Ion Exchange Chromatography. The amino acids measured were alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine and tryptophan. Section 6.3.4 presents the main findings.

6.3.4 Intense mechanical working of DDGS to alter protein structure

The effect of intense mechanical shear stress on DDGS was investigated, the hypothesis being that it might alter the protein structure hence affecting DDGS digestibility. The stress was applied by extrusion and the impact was assessed by Dacron bag tests on live ruminants.

Batches of DDGS with two levels of moisture content, 30% and 38%, were prepared in 5 kg sub-batches using the DDGS supplied by Ensus, which had a 12% moisture content (approx.). The 30% and 38% water content batches were prepared by mixing 1 kg of water with 4 kg of DDGS, and 1.5 kg of water with 3.5 kg of DDGS, respectively, for 45 minutes. The resulting batches were stored in a cold store at 8°C for about 36 hours (batches were prepared on a Monday evening and extrusion took place on the following Wednesday in the morning). The batches were extruded at the Manchester Food Research Centre of the Manchester Metropolitan University using an APV twin screw extruder, shown in Figure 28, with six heating zones on the barrel and an adjustable screw profile.

Batches of DDGS at each water content were extruded without a die at screw speeds of 300 rpm and 500 rpm, and for each speed at barrel temperature of 25°C and 60°C. The barrel temperature was maintained constant across all six heating zones, *i.e.* without a temperature gradient, and the screw profile was maintained across all trials.

In addition to the eight batches above, two more batches were run for each water content, one was recycled through the extruder, *i.e.* extruded twice in succession, and one extruded through a single hole die. For the 30% water content batches, those extrusions were run at 300 rpm and 60°C and for the 38% water content batches, they were run at 300 rpm and 25°C.

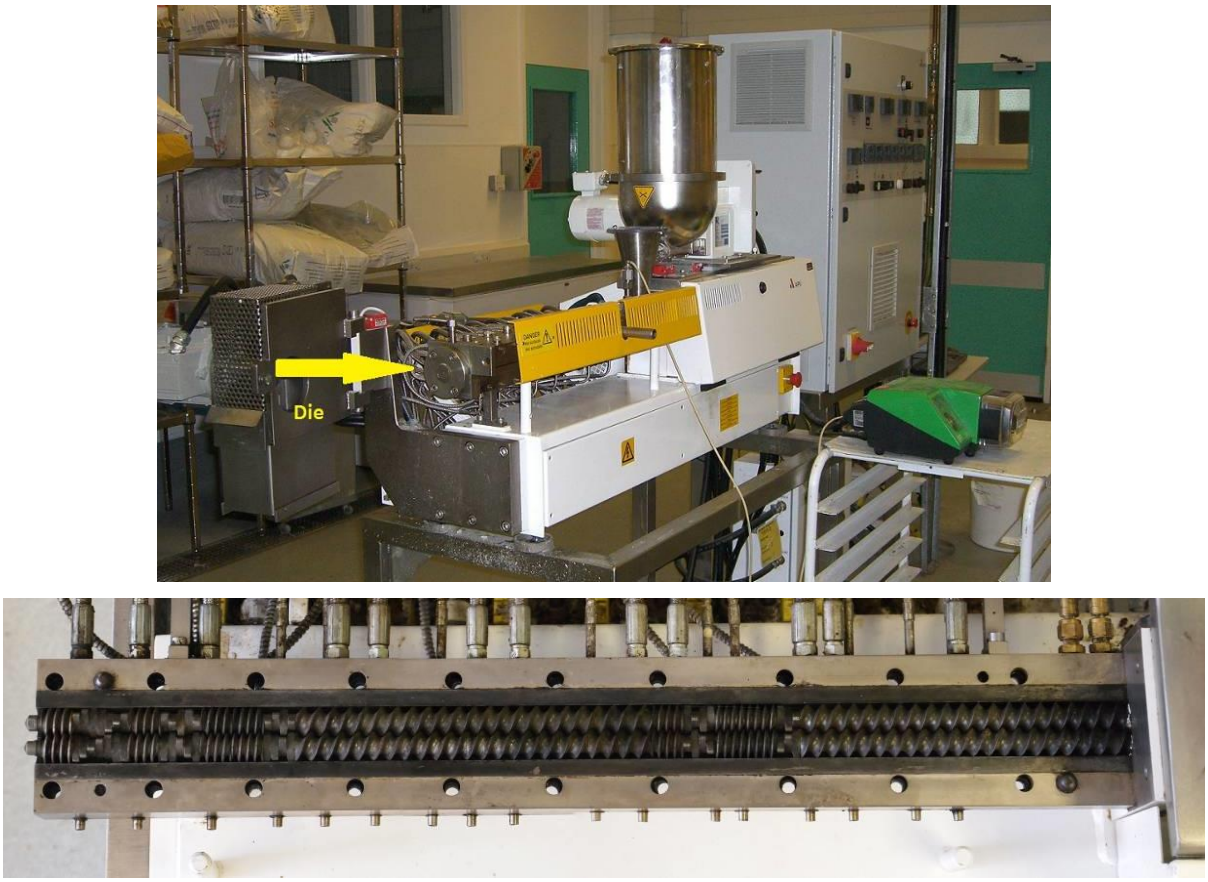


Figure 28 APV twin screw extruder, showing the single hole die attached at the end of the barrel (top), and the screw profile (bottom).

A 600 g sample was taken from each of the eight batches without recycling and die extrusion, and smaller sized samples from the other four batches (double extruded, and extruded through die) because of the limited amount of material. The samples together with a control were kept in a cold store at 8°C until shipped the following day to the University of Nottingham for digestibility tests.

6.3.5 Enzymatic reduction of syrup viscosity

The effect of enzyme treatment on the viscosity of the syrup from the bioethanol production process was investigated. Two samples of syrup were supplied by Ensus in December 2012 and March 2013. The process in December 2012 was using a combination feedstock of 20% Maize and 80% Wheat. By March 2013 the feedstock had changed, information about which was not made available. Thus the two syrups examined had different properties and responded to enzyme treatments differently, but their differing origins were not known and could not be used to inform detailed interpretation. This study is therefore limited to broad interpretations about the scope for enzymatic reduction of syrup viscosity.

Syrup viscosity was measured using a Rapid ViscoAnalyser (RVA, Newport Scientific, Australia) based at the Scotch Whisky Research Institute. The enzymes used are listed later in the Results section; they comprised a range of enzymes from different sources including alpha-amylase, beta-glucanase, a beta-glucanase/xylanase mix, proteases, a hemicellulase, a mixture of saccharifying enzymes, and a bacterial xylanase. The enzymes Endoprotease and Hemicellulase (both from Sigma Aldrich) were in powder form. To prepare a solution of those enzymes, 50 μg of enzyme powder were dissolved in a 50 mL of distilled water in a 100 mL glass beaker. They were then placed on a magnetic stirrer for 15 minutes. The other enzymes were in liquid form.

Syrup was incubated with enzymes by weighing 60 g of syrup into a 100 mL glass beaker and adding 60 μL of enzyme solution (the enzyme dose was decided based on discussion with project partners). The beakers were placed into a waterbath at 55°C, along with a control beaker of syrup with no enzyme added. This temperature was chosen as a compromise between the optimal incubating temperatures for the different enzymes (according to the enzyme supplier's datasheet); constraints on resources, including number of water baths and time, did not allow incubating each enzyme separately at its optimum temperature. pH also affects enzyme activity; the syrup had a pH of 4.35. The syrup samples were incubated in the water bath for two hours, with periodic stirring every 15 minutes with a glass rod (a separate glass rod was used for each beaker to avoid cross contamination). Two hours were chosen due to time constraints, with the time of placing the beakers in the water bath staggered to allow time for RVA testing of each sample. After two hours each sample was removed from the water bath and left for 15 minutes to cool down (15 minutes were chosen because this was the time between placing the different beakers in the water bath).

The viscosity of each sample after incubation was tested in the Rapid ViscoAnalyser, shown in Figure 29, according to the following procedure: The RVA was zeroed according to the manufacturer's procedure (run with no fluid). 20 g of syrup was placed in the aluminium vial of the RVA; this amount was chosen so that the syrup completely covered the paddle. A plastic paddle was placed in the vial and attached to the instrument. The test was then run at a temperature of

40°C and a speed of 100 rpm for five minutes, these values based on preliminary trials investigating the effects of temperature and stirring speed.

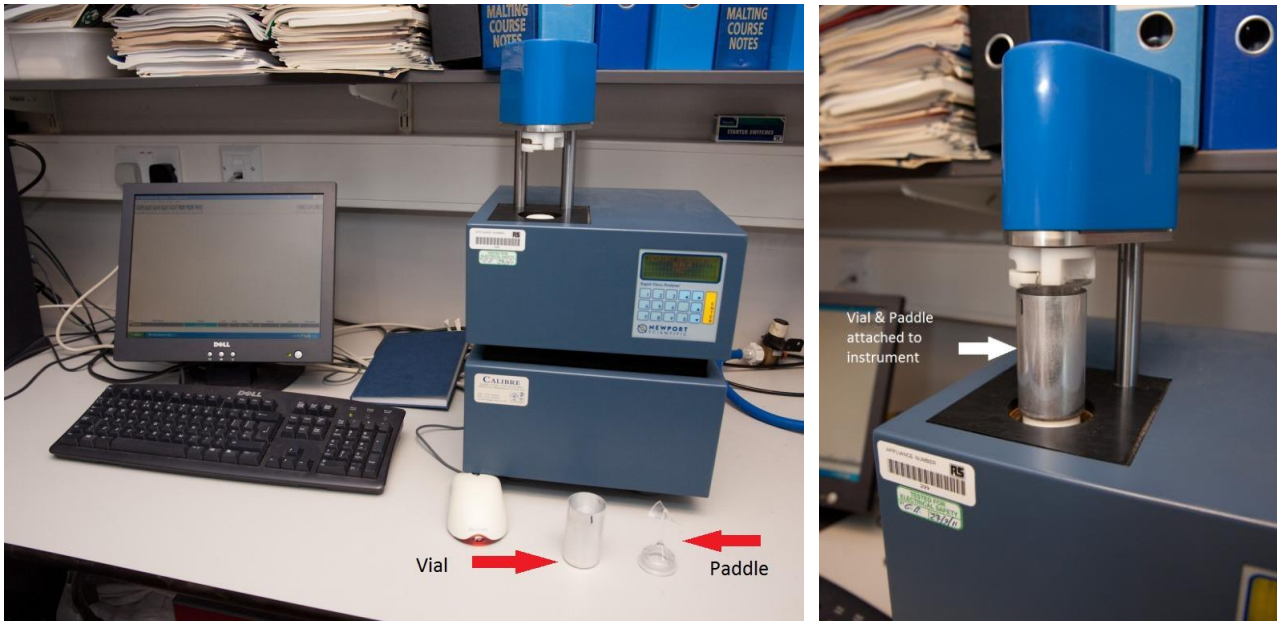


Figure 29 RVA with aluminium vial and plastic paddle on bench (left) and attached to the instrument (right).

6.4 Results

This section describes the results from studies on arabinoxylan extraction from DDGS, dry fractionation of DDGS, fungal fermentation of DDGS and intense mechanical working of DDGS, and interprets these results in terms of their relevance to enhancing the nutritional value of DDGS for animal feed. The effects of enzymatic reduction of syrup viscosity are also described.

6.4.1 Extraction of Arabinoxylans (AX) from DDGS

As noted in Section 6.3.1, in the current work yields of AX were low and time constraints prevented extensive studies to improve extraction, so this work is not reported in detail here. Nevertheless, the AX work still had validity and relevance to the project's aims, which can be summarised as follows:

i) The general principle that processing improvements and product streams should be evaluated within the conceptual framework of an integrated biorefinery is an important principle, and one which the AX example served to highlight – the potential to extract AX from DDGS arises from the co-production of bioethanol, which is used in the AX production process, while the value of removing AX from DDGS is not only that the nutritional value of DDGS could be improved, but that AX has potential as a valuable product in its own right; otherwise its removal would have the effect of reducing the overall quantity of DDGS and hence the overall revenue. This wider context for evaluating AX is discussed further in the Discussion section, Section 6.5.

ii) The specific studies have highlighted issues and challenges in relation to quantifying the AX contents of feedstocks and of extracts, and of performing extractions that give high yields and purities. Work is ongoing in this area, work that has been advanced by the preliminary studies undertaken within this project and that, even if not achieved within the resources and timescales of the current project, has the potential ultimately to benefit the aims of the current project.

Despite the difficulties of several aspects of the AX studies, the procedure for measuring AX contents was applied to samples produced by dry fractionation of DDGS, which is described in the next section.

6.4.2 Dry fractionation of DDGS by sieving and elutriation

Table 67 summarises the investigations to fractionate DDGS using combinations of sieving and elutriation and the fractions produced. The fractions were analysed for Crude Protein, Crude Fibre, Neutral Detergent Fibre, Oil B and Ash.

Table 67 Summary of dry fractionation studies involving sieving and elutriation.

Process	***Fractions produced***	***Codes***
Sieving	Fine, Medium, Coarse	F, M, C
Elutriation at Low (1) and High (2) air flowrates	Light, Medium, Heavy	L1, M1, H1 L2, M2, H2
Sieving followed by elutriation	Light particles of Finely sieved fraction Medium particles of Finely sieved fraction Light particles of Medium sieved fraction Medium particles of Medium sieved fraction Medium particles of Coarse sieved fraction Heavy particles of Coarse sieved fraction	LF MF LM MM MC HC
Elutriation followed by sieving	Fine particles of Light weight fraction Coarse particles of Light weight fraction Fine particles of Medium weight fraction Coarse particles of Medium weight fraction Fine particles of Heavy weight fraction Coarse particles of Heavy weight fraction	FL CL FM CM FH CH
Double elutriation	Light particles of Light weight fraction Medium particles of Light weight fraction Light particles of Medium weight fraction Medium particles of Medium weight fraction Heavy particles of Medium weight fraction Medium particles of Heavy weight fraction Heavy particles of Heavy weight fraction	LL ML LM MM HM MH HH

Figure 30 shows the cumulative particle size distribution (psd) of the original DDGS and compares this with the psd of Maize DDGS reported in the literature (Srinivasan *et al.*, 2008). Clearly the Wheat DDGS used in the current work comprised much larger particles, with an x_{50} (size below which 50% of the particles fall) of around 1500 μm compared with 500 μm for the maize. If the Elusieve process that applies sieving and elutriation to Maize DDGS is considered to be able to generate valuable differentiation, it might be hoped that the wider psd of the Wheat DDGS should make this even more the case (however, as discussed later, the larger Wheat DDGS particles are agglomerates that tend towards the average composition of the component particles).

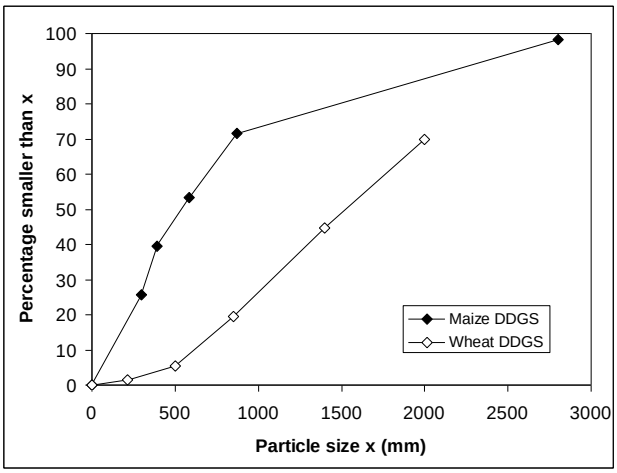


Figure 30 Particle size distribution of the Wheat DDGS used in the current work, and of Maize DDGS reported by Srinivasan *et al.* (2008).

Based on this psd, the DDGS was divided into three fractions separated at 2000 and 850 μm, yielding 19.5% Fine, 49.5% Medium and 31% Coarse material. Meanwhile, elutriation at High and Low air flowrates was also used to separate the material into three fractions, Light, Medium and Heavy. Figure 31 shows the proportions of the three fractions produced by sieving or by elutriation with High or Low air flowrates. As expected, the Low air flowrate lifted less material than the High air flowrate, resulting in a smaller Light fraction and greater Heavy fraction.

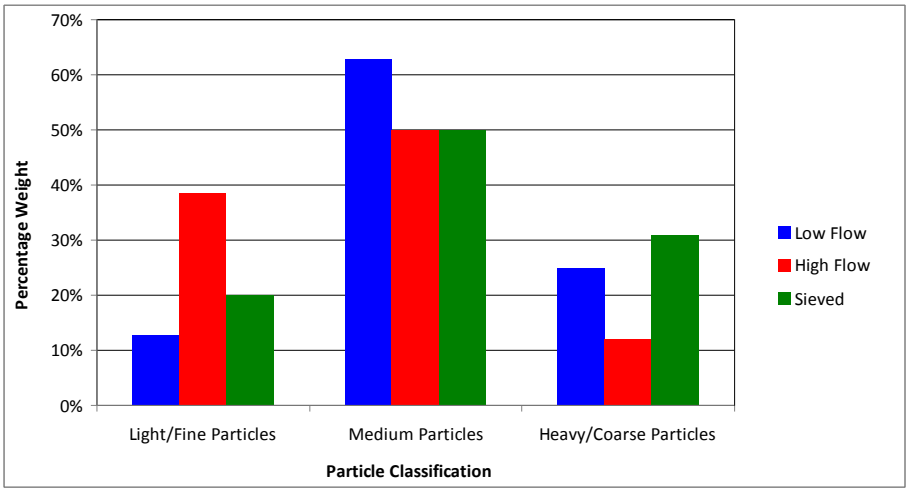


Figure 31 Proportions of the three fractions produced by sieving (Fine, Medium, Coarse) or by elutriation with High or Low air flowrates (Light, Medium, Heavy).

Figure 32 shows the crude protein and crude fibre contents (on a 10% moisture basis) of the fractions produced by the three fractionation approaches. Clearly larger particles, whether the Heavy particles following elutriation or the Coarse particles produced by sieving, had higher protein contents and lower fibre contents. This indicates that, despite the homogenising effect of agglomeration during drying, DDGS particles do vary compositionally, and that compositionally

distinct fractions can be produced by dry fractionation. The original DDGS had a crude protein content of 32.7% and a crude fibre content of 8%. Fractionation by sieving gave a coarse fraction enriched in protein to 33.9% and depleted in crude fibre to 7.24%, and a fine fraction with protein and fibre contents of 31.9% and 9.25%, respectively. Elutriation was able to give greater levels of differentiation, particularly under the Low flowrate which produced a heavy fraction enriched in protein to 34.9% and depleted in crude fibre to 6.74%, and a light fraction with protein and fibre contents of 30.1% and 9.69%.

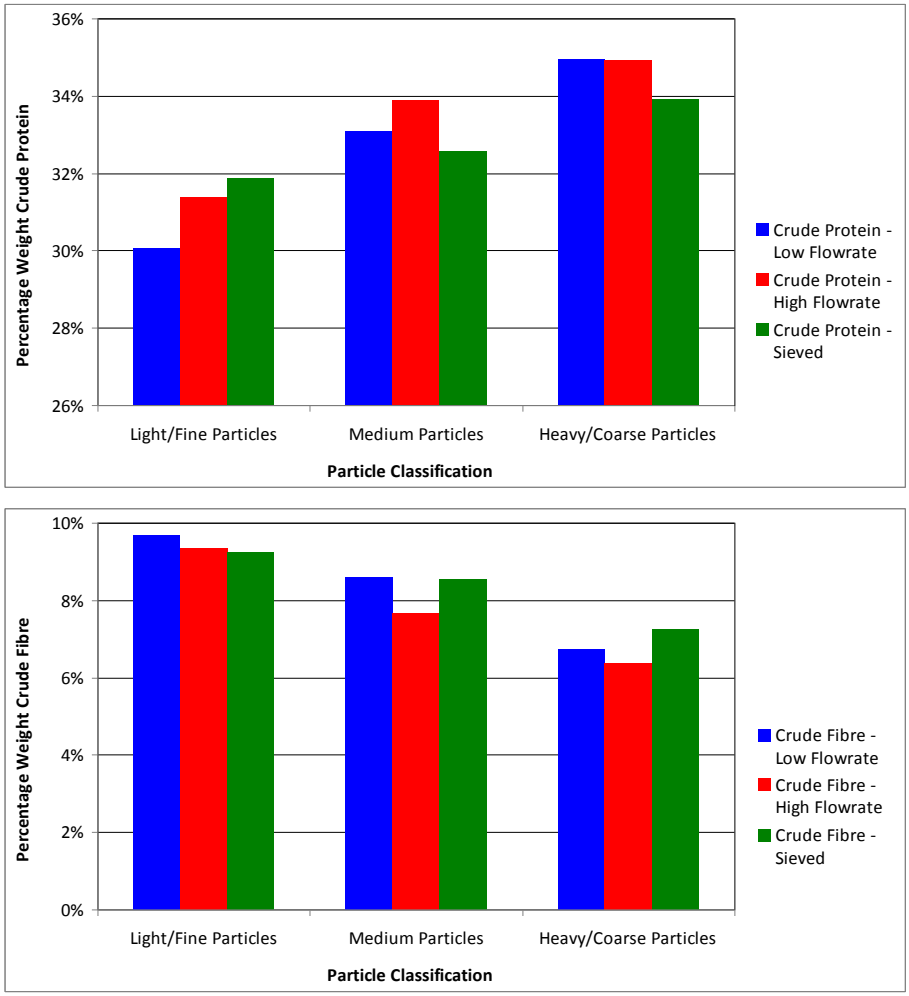


Figure 32 Crude protein (top) and crude fibre (bottom) contents on a 10% moisture basis for the DDGS fractions produced by Sieving and by Elutriation at High and Low air flowrates.

(At first glance it may seem strange, and indeed impossible, that going from High to Low air flowrate could increase the crude fibre content of all three fractions! However, this is indeed possible and is not a result of experimental or computational error. It is an example of the Will Rogers phenomenon, after the Oklahoma comedian who joked that Oklahomans who move to California raise the average IQ of both states. From Figure 7.7, going from High to Low air flowrate increases the Heavy and Medium fractions and reduces the Light fraction – in other words,

material previously separated as Light now falls into the Medium fraction, and material previously contributing to Medium now contributes to the Heavy fraction. The Light material in general has the highest fibre content. Moving from a High to a Low flowrate means that only the Very Light material (which has an even higher fibre content) is now lifted into the Light fraction, increasing its average fibre content. Meanwhile, the Somewhat Light material with a somewhat high fibre content moves to the Medium fraction with its moderate fibre content, thus increasing the average fibre content of this fraction. This is enhanced by some lower fibre material moving out of the Medium fraction and into the very-low-fibre Heavy fraction, also serving to increase the average fibre content of both fractions.)

The results indicate that smaller and lighter particles contain more fibre, and larger and heavier particles contain more protein. Bran (or even residual husk) material from the wheat is higher in fibre and likely to form light, flat, low density particles that would be easily lifted by elutriation, so it makes sense that the Light material was high in fibre. Figure 33 shows SEM micrographs of Light and Heavy particles, illustrating the more globular and agglomerated nature of the Heavy particles, comprising distinct components “glued” together, while the Light particle is a single large flat piece of probably bran tissue, which would clearly be more conducive to being lifted in an air flow than the Heavy particle.

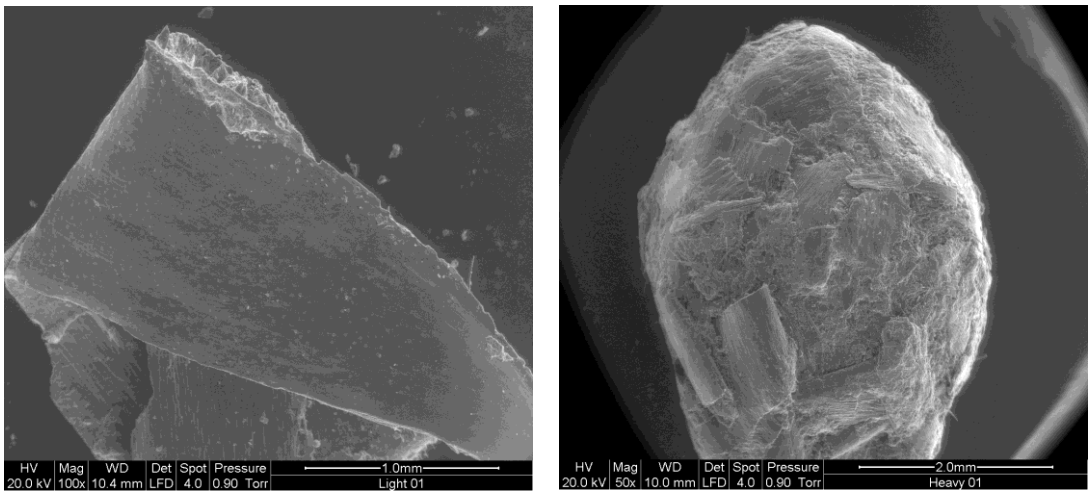


Figure 33 SEM micrographs of examples of Light and Heavy particles.

The differences in protein and fibre content achieved by fractionation in the current work are relatively small and too small to be of commercial relevance, but the principle is established that dry fractionation can produce streams varying in composition. Optimisation of the dry fractionation could yield even more compositionally distinctive streams. Even then, the value of DDGS is mostly in its protein content; on the face of it, producing a protein-rich DDGS stream of higher value is at the expense of a protein-depleted stream of lower value, resulting in no net benefit – a zero-sum

game. However, at a more abstract level, fractionation is in general the basis for process integration and adding value; the potential of fractionation is in what other benefits it can yield in an integrated biorefinery. The previous section was about arabinoxylan extraction; it is conceivable that a very high fibre fraction produced from fractionation and containing minimal protein could be a suitable stream for arabinoxylan extraction, such that in the overall context of the integrated biorefinery, the fractionation allows a more targeted approach to processing that is more effective and economic.

Meanwhile, at the practical level, the effectiveness of the separation is compromised by the nature of the DDGS used. The addition of the Solubles fraction (the S of DDGS) to the Wetcake (the DDG) causes agglomeration of particles during drying, such that inevitably the composition of agglomerates tends towards the average composition. Drying just the DDG is likely to produce more compositionally distinct particles that can be separated into more compositionally distinct fractions. The current set-up at the Ensus plant did not allow recovery of Wetcake that could be dried without the inclusion of the syrup in order to test this hypothesis in the current project.

Sieving followed by elutriation

The Elusieve process employs sieving followed by elutriation to give better protein-fibre separation for Maize DDGS (Srinivasan *et al.*, 2008, 2009); the same approach was investigated in the current work. Table 35 shows how each sieve fraction then elutriated at the High air flowrate into Light, Medium and Heavy material. Elutriating at the High air flowrate separated the Coarse and Medium fractions reasonably evenly; the Medium fraction after sieving contained around 37% light particles that ended up in the Light fraction following elutriation, and 59% in the Medium fraction, with a few Heavy particles, while the Coarse fraction after sieving resulted in 53% Medium particles and 43% Heavy particles after elutriation, with a few Light particles. However, the Fine fraction ended up almost entirely (94%) in the Light fraction following elutriation. In order to get a more even split, the Fine fraction was therefore elutriated at the Low air flowrate, giving 46% Light particles and 52% Medium, as also shown in Figure 34. Thus six fractions were produced, the three sieve fractions each separated into two fractions by elutriation. These samples were sent for analysis of protein and crude fibre.

Figure 35 shows the crude protein and fibre contents on a 10% moisture basis for the fractions following sieving and elutriation. As before, the Coarse fractions were richer in protein and depleted in fibre, and vice versa for the Fine fractions. Elutriation served in each case to accentuate the differences, but not greatly. The benefit of elutriation appeared greatest on the Middle fraction from sieving, resulting in a Light fraction with a protein content of 29.7%, slightly lower (but probably not significantly) than the lowest value achieved previously of 30.1%, and a crude fibre content of 9.47%. The highest protein/lowest fibre combination was 34.1%/6.4%,

similar to that obtained previously from elutriation on its own, which produced a heavy fraction enriched in protein to 34.9% and depleted in crude fibre to 6.74%, and a light fraction with protein and fibre contents of 30.1% and 9.69%. Thus in the current work, sieving followed by elutriation did not result in noticeably enhanced fractionation.

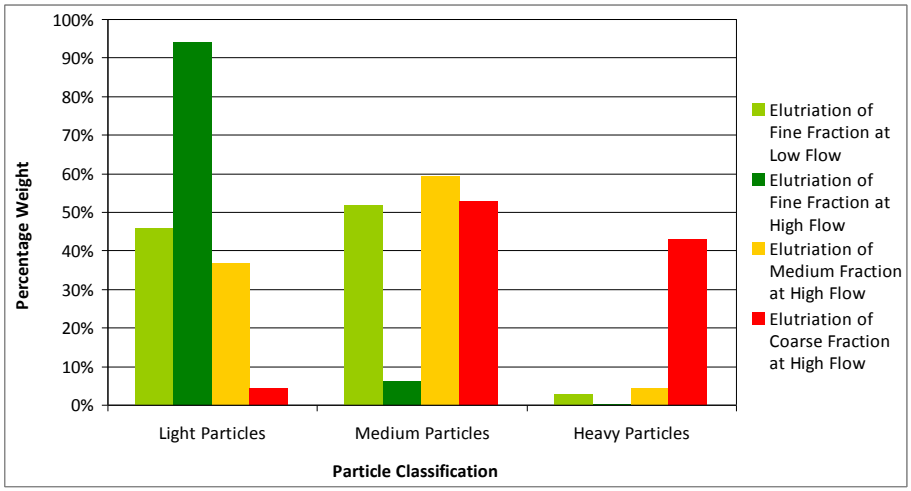


Figure 34 Particle classification of DDGS fractions obtained by sieving followed by elutriation at a High air flowrate, plus the classification for elutriation of the Fine fraction at a Low air flowrate.

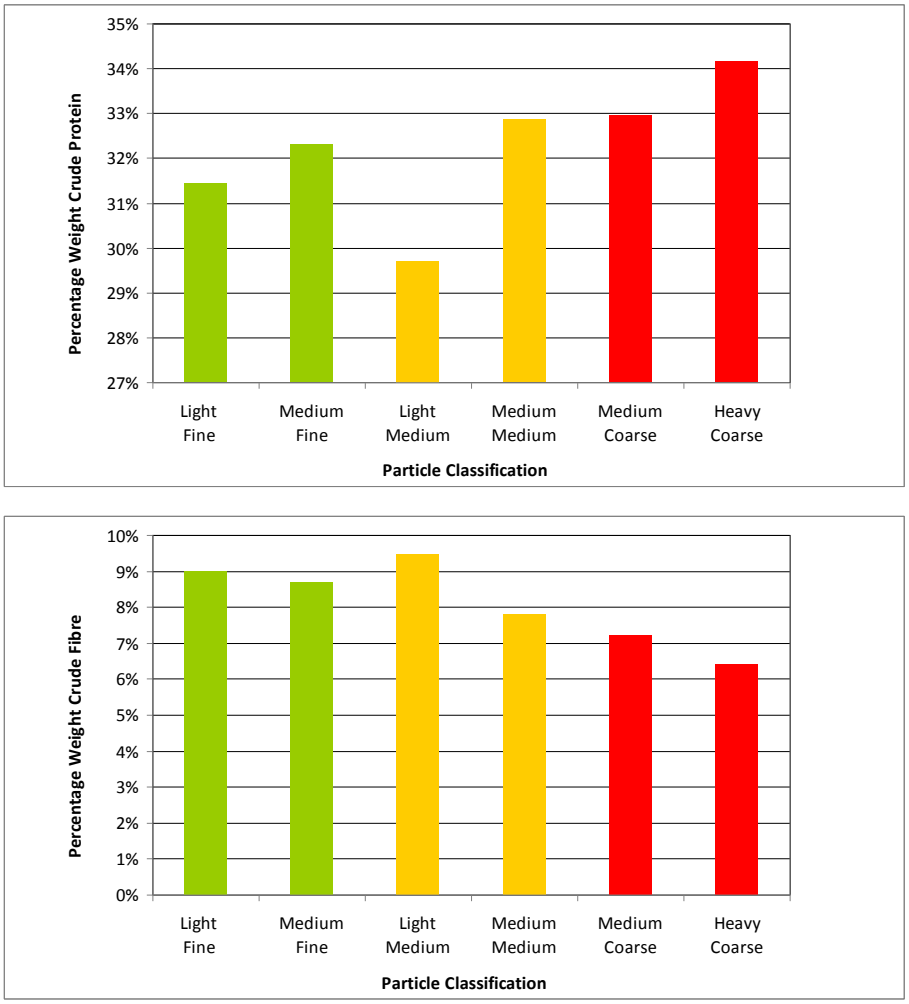


Figure 35 Crude protein (top) and Crude fibre contents (10% moisture basis) for DDGS fractions obtained by sieving followed by elutriation.

Elutriation followed by sieving

Figure 36 shows the particle size distribution of the original DDGS and of the three fractions obtained following elutriation in the dehusker at the High air flowrate. Clearly the Heavy fraction was dominated by large particles, as was the Medium fraction to a lesser extent, while the Light fraction contained a wide range of particles (this reflects that there are more ways of being "Light" than of being "Heavy" – Heavy particles were probably all large agglomerates of similar structure and composition, whereas Light material could encompass the range from small dense spheres to large flat particles). Based on these results, each fraction was then divided into two fractions of almost equal weight; the Light fraction was separated into two fractions using a sieve of 850 μm, the Medium fraction using a sieve of 2000 μm and the Heavy fraction using a sieve of 3150 μm.

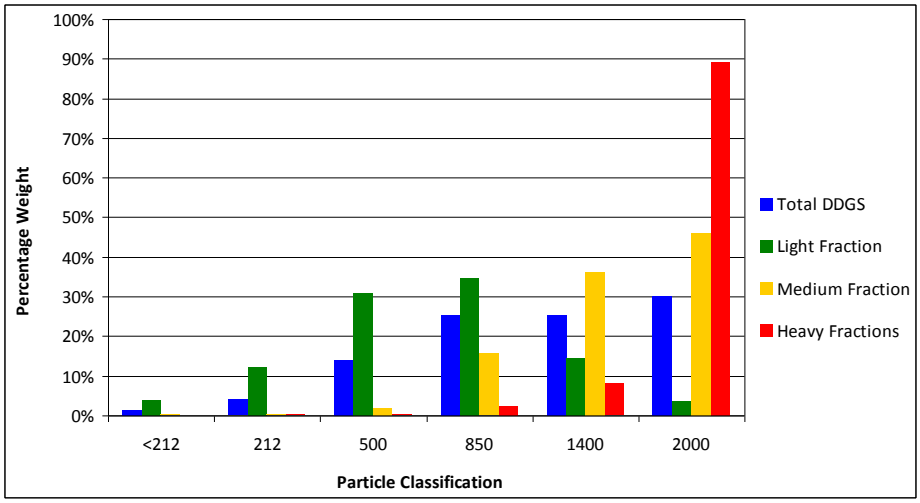


Figure 36 Particle size distribution of DDGS and of fractions obtained by elutriation at high air flow rate followed by sieving.

Figure 37 reports the crude protein and crude fibre contents of the Fine and Coarse size fractions from the Light, Medium and Heavy elutriated fractions. The degree of separation achieved was greater than for the previous results; in this case, the Coarse particles from the Light fraction had a much lower protein content of 28.0% and a crude fibre content of 9.81%, making these the most highly differentiated samples achieved. At the other end of the scale, the Fine particles from the Heavy fraction has the highest protein content, 34.3% and the lowest crude fibre content, 6.38%, similar to fractions produced previously. Meanwhile, it is interesting to observe that the effect of sieving on the Medium fraction was opposite to the other two, reflecting differences in the natures of the particles that fall into the three fractions. Again, the differences in composition are not dramatic and too small to be commercially relevant, but the work underlines that there are various dry fractionation strategies that could be deployed to create defined fractions. Once again it is noted that a feedstock that excluded the Solubles may be more amenable to dry fractionation.

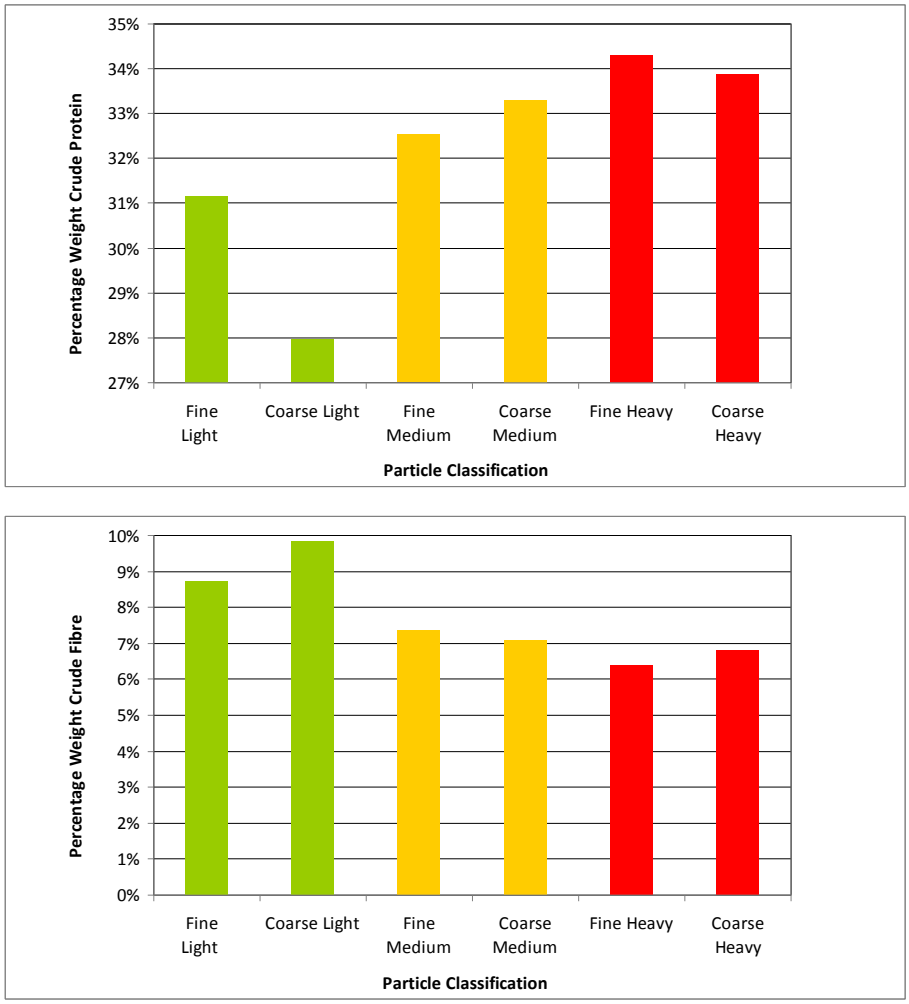


Figure 37 Crude protein (top) and Crude fibre contents (10% moisture basis) for DDGS fractions obtained by elutriation followed by sieving.

Multiple elutriations

DDGS was separated into three fractions by elutriation in the dehusker at the High air flowrate. Each fraction was then elutriated again at the High flowrate. This resulted in seven new fractions: the Light fraction produced Light and Medium particles on further elutriation, but (practically) no Heavy particles; the Heavy fraction produced Heavy and Medium particles, but no Light particles, and the Medium fraction yielded Light, Medium and Heavy particles.

Figure 38 shows the distribution of particles following double elutriation. Elutriating the Light fraction again resulted in 90% of the particles still being lifted into the Light fraction, with nearly 10% creating a new Middle fraction, plus a few particles ending up in the Heavy collection tray (too few to form a fraction worth analysing). 75% of the Medium particles stayed in the Medium fraction, while 12% were lifted to create a new Light fraction, and 13% formed a new Heavy fraction. Meanwhile, 73% of the original Heavy fraction stayed as Heavy particles, while 26%

formed a new Medium fraction, and a few were lifted into the cyclone (again, too few to form a fraction). Thus seven new fractions were created from the double elutriation.

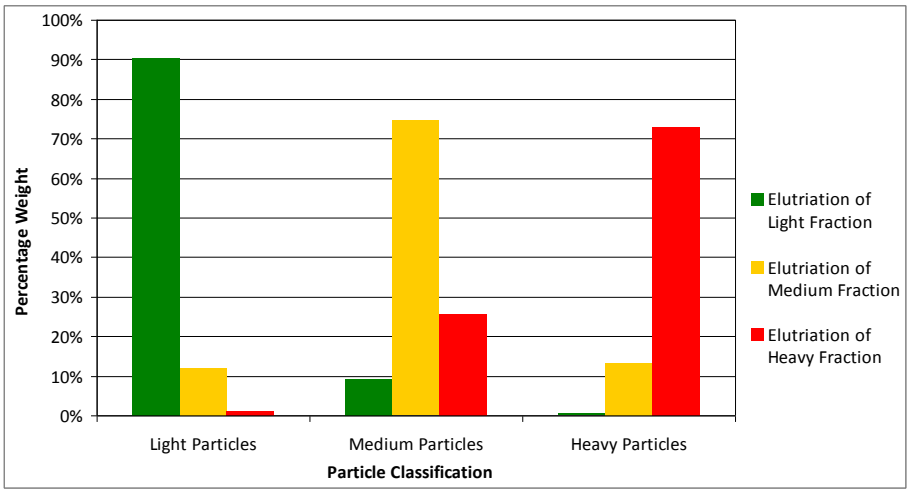


Figure 38 Particle classification of DDGS fractions obtained by double elutriation at a High air flowrate.

Figure 39 shows the crude protein and fibre contents of the seven fractions produced from double elutriation. (Colours, and the second letter of the codes, indicate the original separation into Light, Medium and Heavy; the first letters of the codes indicate fractions resulting from the second elutriation, as given in Table 38, *e.g.* ML is the Medium fraction produced from the original Light fraction.) Once again, heavier particles are richer in protein, lighter particles richer in fibre, with the exception of the original Heavy fraction which separated into new Medium and Heavy fractions with similar compositions. These results again indicate that double fractionation has the potential for greater compositional separation, but once again the enhancements are small; the principle is again established that dry fractionation could be implemented in several ways to produce compositionally distinct fractions, but the current process for producing DDGS at the Ensus plant limits the extent of enrichment that can be achieved.

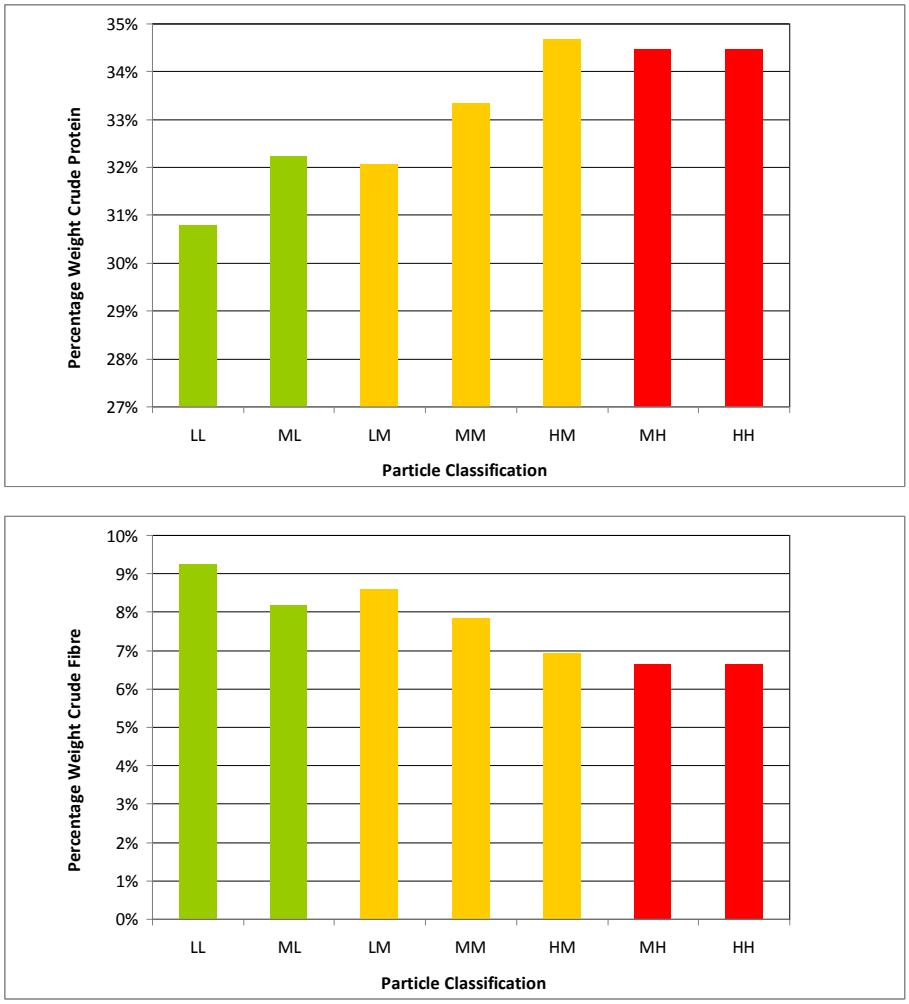


Figure 39 Crude protein (top) and Crude fibre contents (10% moisture basis) for DDGS fractions obtained by double elutriation.

The results presented in this section are based only on the crude protein and crude fibre; Appendix 1 presents the complete results for Neutral Detergent Fibre (NDF), Oil B and Ash. In the above trials, Elutriation followed by Sieving arguably gave the greatest differentiation of fractions; Figure 40 presents the Neutral Detergent Fibre (NDF), Oil B and Ash results from those trials. The NDF results broadly follow those of the Crude Fibre, showing the Coarse Light fraction as having the greatest fibre content, with some difference in the details around the Medium fraction, while Oil B and Ash contents did not vary dramatically between fractions.

In general, this work has established that dry fractionation, which has been applied to differentiate fractions from Maize DDGS, could also be applied to Wheat DDGS. The degree of separation achieved in the current work was too small to be of commercial relevance, but could be increased if applied to a more suitable feedstock, *i.e.* one in which the Solubles fraction has been omitted, in order to avoid production of uniform agglomerates during drying. The production of compositionally

distinct fractions is likely only to be of economic benefit if carried out within an integrated biorefinery in which targeted further processing of the fractions allows value to be added.

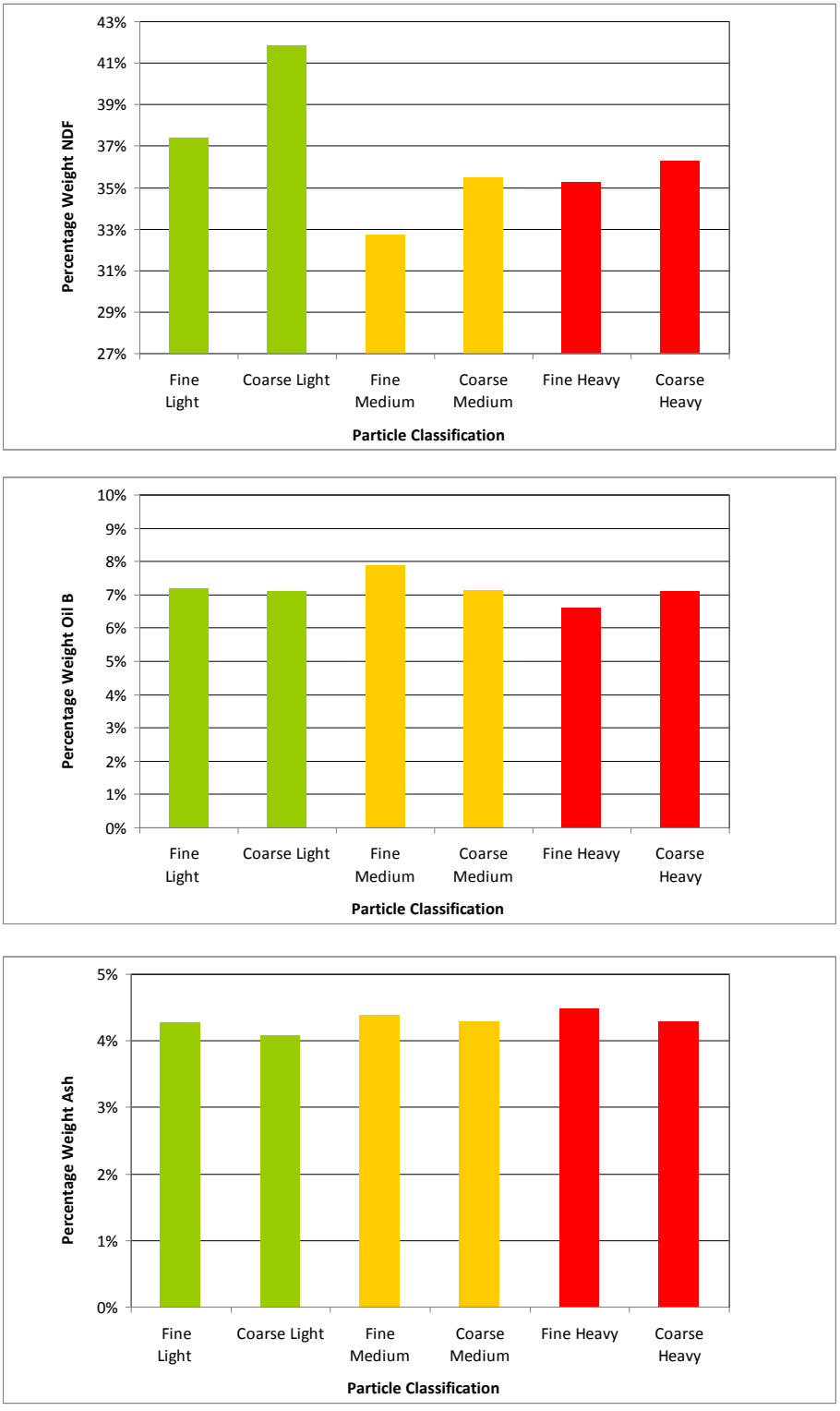


Figure 40 Neutral detergent fibre (top) and Oil B (middle) and Ash (bottom) contents (10% moisture basis) for DDGS fractions obtained by elutriation followed by sieving.

Arabinoxylan contents of sieved and elutriated fractions

In a separate study carried out as part of an MSc project, DDGS was fractionated by either sieving or by elutriation as described above, and the arabinoxylan contents of the fractions measured as described in Section 6.3.1.

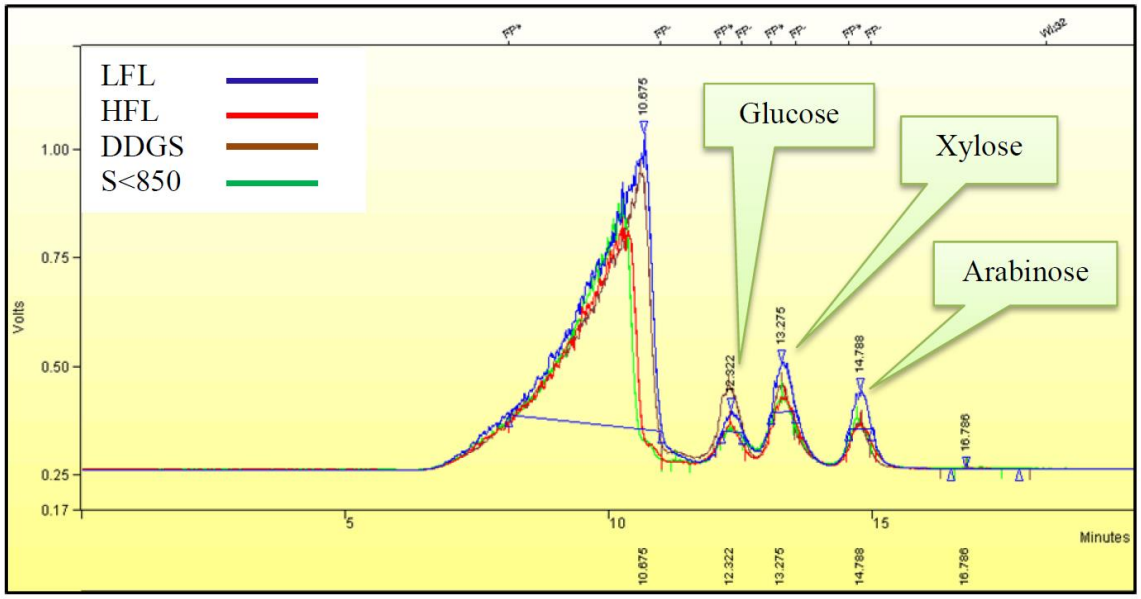


Figure 41 HPLC traces of hydrolysed samples of DDGS and of Light fractions from the elutriation at Low (LFL) and High (HFL) air flowrates, and from the Fine fraction obtained from sieving.

Table 68 reports the arabinose and xylose contents of the dry fractionated samples and the total AX contents as a percentage of dry weight, along with the A:X ratio. There is a consistent suggestion that the larger particles (Heavy fractions from elutriation and the Coarse fraction from sieving) had lower AX contents, while the highest AX content was for the Light fraction from the Low air flowrate. This is consistent with the picture from the earlier studies that the Light particles contain more fibre and hence more AX. This fraction also had the highest A:X ratio, implying a more substituted arabinoxylan and illustrating the point that AX from different fractions may differ not just in quantity but in structure and hence functionality. AX has substantial potential as a co-product of bioethanol and DDGS production, but substantial challenges remain in terms of determining the functionality of different types of AX for different uses, and hence the optimal source of the AX and, having identified this, suitable fractionation and extraction processes within an integrated biorefinery.

Table 68 Arabinose, Xylose, Total AX and A:X ratio of DDGS and fractions produced by sieving or by elutration.

Samples	Arabinose% db	Xylose% db	Total AX Content %db	A:X ratio
DDGS	6.34	10.95	15.21	0.579
Sieved fractions:				
S2000 µm (Coarse)	5.74	10.45	14.24	0.549
S850 µm (Medium)	6.68	12.12	16.55	0.551
S<850 µm (Fine)	6.53	11.19	15.59	0.584
Low air flow:				
Heavy (LFH)	5.71	10.02	13.84	0.570
Medium (LFM)	6.71	11.70	16.20	0.574
Light (LFL)	7.38	11.86	16.93	0.622
High air flow:				
Heavy (HFH)	5.68	9.70	13.54	0.586
Medium (HFM)	5.61	9.94	13.69	0.564
Light (HFL)	6.47	12.03	16.28	0.538

6.4.3 Fungal fermentation of DDGS to alter its amino acid composition

Solid state fermentation of DDGS was carried out using the fungi *Aspergillus oryzae* and *Aspergillus awamori*, and the resulting amino acid compositions measured and compared with the original DDGS. This work was carried out via an HGCA-funded undergraduate studentship reported in Chawla *et al.* (2013); the main findings are summarised briefly here. Table 69 summarises the trials performed.

Table 69 Summary of DDGS fermentation experiments carried out for amino acid profiling

	System	Experiment Code	Water content (%)	Inoculum size (µL)	Other conditions
1	*A. oryzae*	AO30	30	800	Mixed & unsealed
2	*A. oryzae*	AO40	40	800	Mixed & unsealed
3	*A. awamori*	AA30	30	800	Mixed & unsealed
4	*A. awamori*	AA40	40	800	Mixed & unsealed
5	Mixed culture	AOA1	40	400 of each	Un-mixed & sealed
6	Mixed culture	AOA2	40	400 of each	Mixed & unsealed

The fermented samples were analysed for their amino acid profiles by Sciantec Analytical Services Ltd. using Ion Exchange Chromatography. The amino acids measured were alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine and tryptophan. Figure 42 presents the

amino acid profiles in terms of relative proportions of the overall amino acid content. The majority of the amino acids increased post fermentation, including the essential amino acids, principally at the expense of glutamic acid and phenylalanine which both decreased. In the case of glutamic acid, the decrease may be because during metabolism, glutamic acid undergoes a process of transamination which results in its breakdown to form new amino acids.

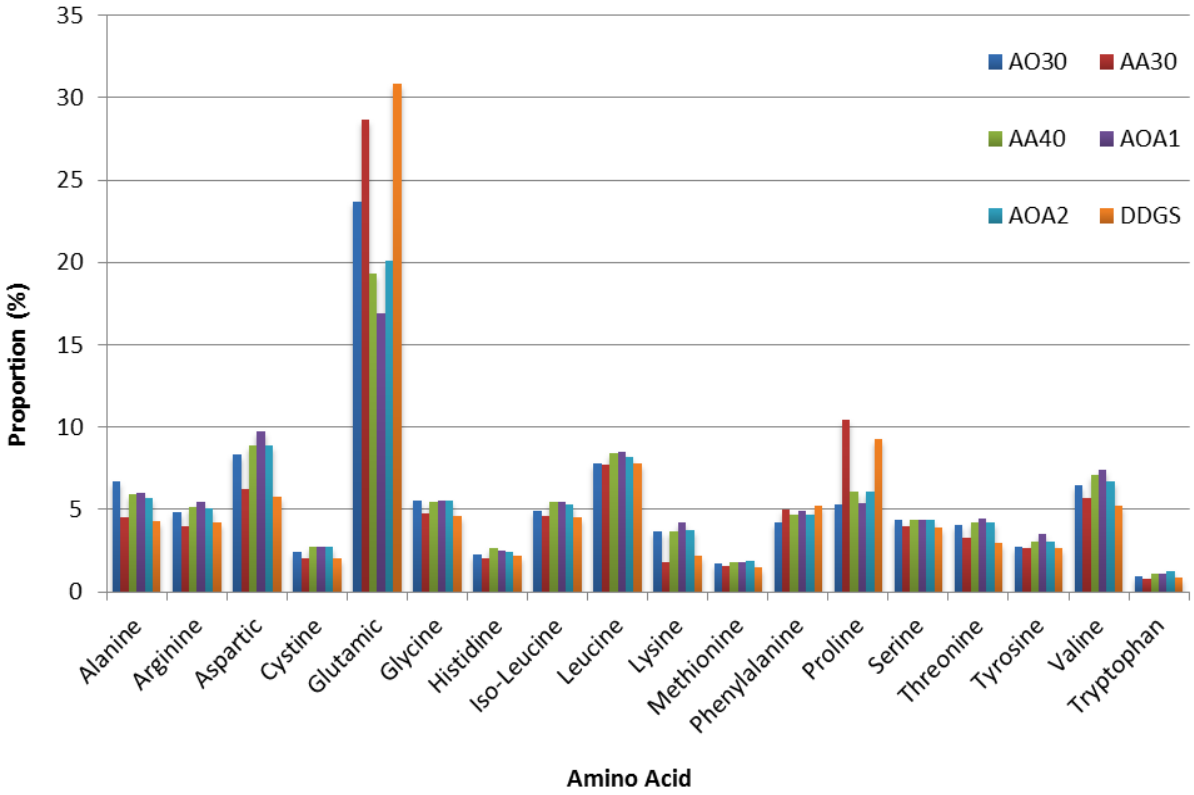


Figure 42 Proportions of different amino acids in fermented samples and unfermented DDGS.

Figure 43 shows the principal component analysis bi-plot of the fermented samples and unfermented DDGS and their relative variance with respect to the individual amino acids. Principal component 1 (PC1) accounts for 96% variation in the data and hence similar samples can be clustered together across the horizontal axis and separated from other dissimilar clusters. The clustering of samples, as indicated by ovals on Figure 43, suggests that AA30 and DDGS are more or less similar to each other in terms of their amino acid profile in comparison to the rest of the samples. Inspection of the raw data in Figure 43 confirms this similarity; samples AA30 and DDGS have similar and noticeably higher proportions of glutamic acid and proline than the other samples, and correspondingly similar and lower proportions of alanine, arginine, aspartic acid, cystine, glycine, histidine, isoleucine, lysine, methionine, serine, threonine, valine and tryptophan (with the evidence from leucine, phenylalanine and tyrosine similar but less clear).

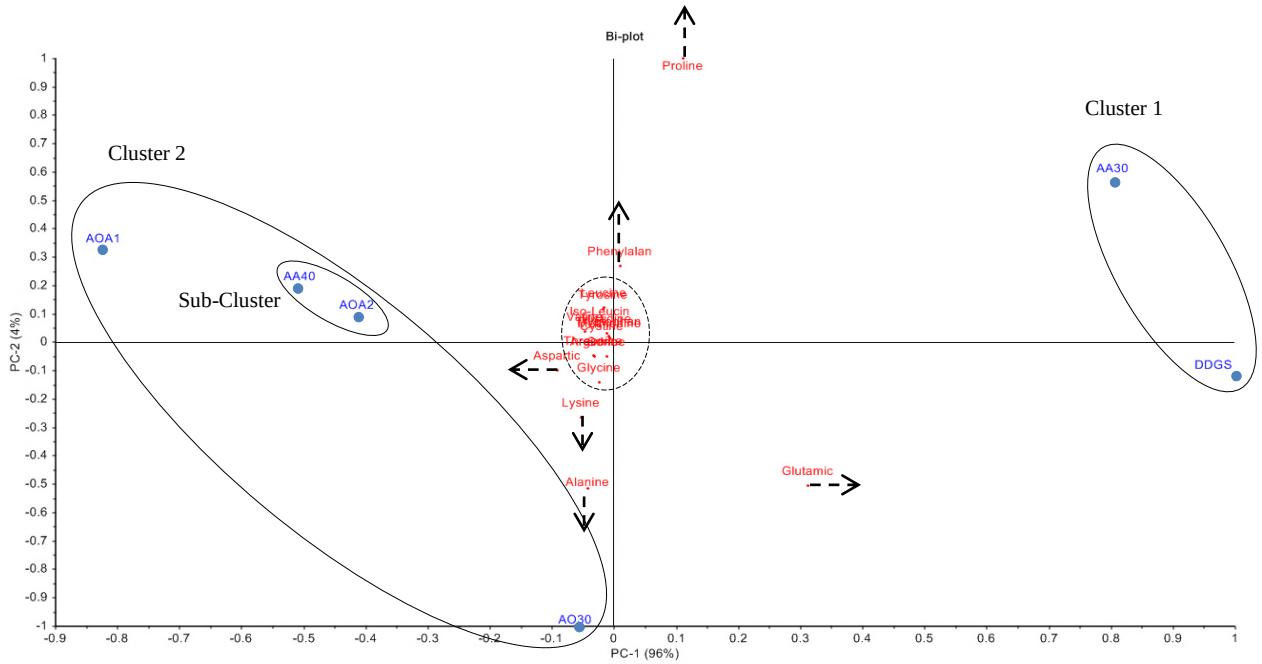


Figure 43 Principal Component Analysis bi-plot for fungal fermentation of Wheat DDGS.

Meanwhile, sample AOA1 is furthest away from the original DDGS indicating that AOA1 attained the greatest variation in its amino acids profile post-fermentation. Samples AO30, AA40 and AOA2 are also substantially different from the unfermented DDGS, the latter two falling closely on the PCA bi-plot. The arrows in Figure 43 represent the load with which each amino acid pulls the samples across the PC bi-plot. Hence, one can conclude that samples in Cluster 2 differed from DDGS principally in terms of increased lysine and alanine and decreased glutamic acid and proline. This can again be confirmed by the data in Figure 42.

Essential amino acids for most animals are arginine, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan. Of these only lysine is clearly distinguishable on the PCA bi-plot, in a position that indicates fermentation led to a greater proportion of lysine, which would be a favourable outcome for improving the nutrition. To look at this more broadly, the ratio of essential to total amino acids (E:T) was calculated for each fermented sample and compared to that of unfermented DDGS. The calculations are reported in Table 70.

Table 70 Ratio of Essential to Total (E:T) amino acids for fermented samples and unfermented wDDGS.

Sample	E:T
AO30	0.41
AA30	0.37
AA40	0.44
AOA1	0.46
AOA2	0.44
DDGS	0.36

According to the ratios calculated in Table 70, the E:T ratio improved for all fermented samples in comparison to unfermented DDGS. This indicates that the proportion of essential amino acids increased within all the fermented samples, thereby supporting the conclusion that solid state fermentation using *Aspergillus spp.* can improve the amino acids profile of DDGS. The trends observed within the E:T ratios of different fermented samples coincide with the ones noted from PCA analysis, thereby adding confidence to this interpretation.

This study demonstrated that solid state fermentation using *Aspergillus spp.* has the potential to alter the amino acids profile of DDGS, to enhance the proportion of essential amino acids. Mixed-culture samples appeared to perform better than mono-culture samples, and *Aspergillus awamori* seemed to have performed better than *Aspergillus oryzae* in the current work. The work has established that enhancing wheat DDGS by solid state fermentation could improve the nutritional quality of DDGS in terms of its amino acid composition.

6.4.4 Intense mechanical working of DDGS to alter protein structure

Table 71 summarises the details of the extrusion trials. Figure 44 illustrates the DDGS exiting the extruder, which appeared no different from the raw DDGS, suggesting at the time that extrusion may have had little effect; nevertheless, the samples were sent to the University of Nottingham for digestibility measurement using Dacron bag studies to measure Dry Matter (DM) and Nitrogen (N) degradability. Degradability is modelled using an exponentially decaying curve:

$$DG = a + b\left(1 - e^{-ct}\right)$$

where *DG* is degraded (disappeared) DM or N at time *t*, *a* is the intercept which represents the rapidly soluble fraction, *b* is the asymptote which represents the potentially degradable fraction, and *c* is the rate of degradation. Effective degradability is calculated as:

$$\text{Effective degradability} = a + \frac{bc}{(c+k)}$$

where *k* is the outflow rate, assumed for dairy cows to be 0.08 h^{-1} (*i.e.* 8% per hour).

Table 71 DDGS extrusion trials and resulting Dry Matter and Nitrogen Degradability.

Sample no.	Water Content %	Temp. °C	Screw Speed rpm	Remarks	Dry Matter Degradability	Nitrogen Degradability
1	12	-	-	Untreated DDGS	0.526	0.386
2	30	25	300		0.800	0.856
3	30	25	500		0.816	0.854
4	30	60	300		0.783	0.818
5	30	60	500		0.785	0.817
6	38	25	300		0.821	0.844
7	38	25	500		0.832	0.843
8	38	60	300		0.793	0.794
9	38	60	500		0.804	0.799
10	30	60	300	Extruded through die	0.790	0.815
11	30	60	300	Recycled (run twice)	0.801	0.852
12	38	25	300	Extruded through die	0.817	0.846
13	38	25	300	Recycled (run twice)	0.832	0.858

Figure 44 DDGS being extruded through the twin screws and collected in a foil tray.

The Effective DM and N degradability of the extruded samples are reported in Table 71 and plotted in Figure 45, where blue represents 30% moisture and red 38% moisture, darker colours represent extrusion at 25°C and the lighter colours represent extrusion at 60°C. Clearly extrusion had a dramatic effect on DDGS degradability as measured by Dacron bag studies in ruminants; DM degradability increased from 0.526 for the original DDGS to an average of 0.806 for the extruded samples, and N degradability increased from 0.386 for the original DDGS to an average of 0.833 for the extruded samples. Despite visual observation not revealing any obvious changes in the DDGS, clearly extrusion resulted in structural changes that dramatically influenced the accessibility of the DDGS during digestion.

Samples 2-9 represent a 2^3 factorial experiment with two moisture contents (30% – samples 2-5 in blue – and 38% – samples 6-9 in red), two temperatures (25°C, represented by the lighter bars, and 60°C represented by the darker bars) and two screw speeds (300 rpm, for the left bar in each pair, and 500 rpm which is the right bar of each pair). It is evident from inspection of the graphs, and confirmed by analysis of variance (ANOVA), that DM degradability and N degradability were both higher when extrusion was carried out at the lower temperature; possibly the high temperature rendered the DDGS less viscous such that less work was applied during extrusion. DM degradability was slightly higher for the 38% moisture samples, although this pattern was not so evident for N degradability. Screw speed appeared to have little overall effect.

Samples 10-13 represent more severe processing, either by processing the DDGS twice, or by extruding through a die (which increases the pressure during extrusion). Extruding twice had more effect than extruding through a die, although neither seemed to increase degradability beyond what was achieved by single extrusions without a die.

The results indicate that intense mechanical working of DDGS can substantially alter its digestion behaviour, and that extrusion is a mechanism by which such mechanical work could be applied, with the details of the extrusion conditions influencing the change in digestibility. For ruminants, this dramatic increase in digestibility is probably not a good thing; the hope had been to increase Rumen Bypass Protein which would have decreased the measured digestibility. However, the increased digestibility could be relevant to feeding DDGS to non-ruminants.

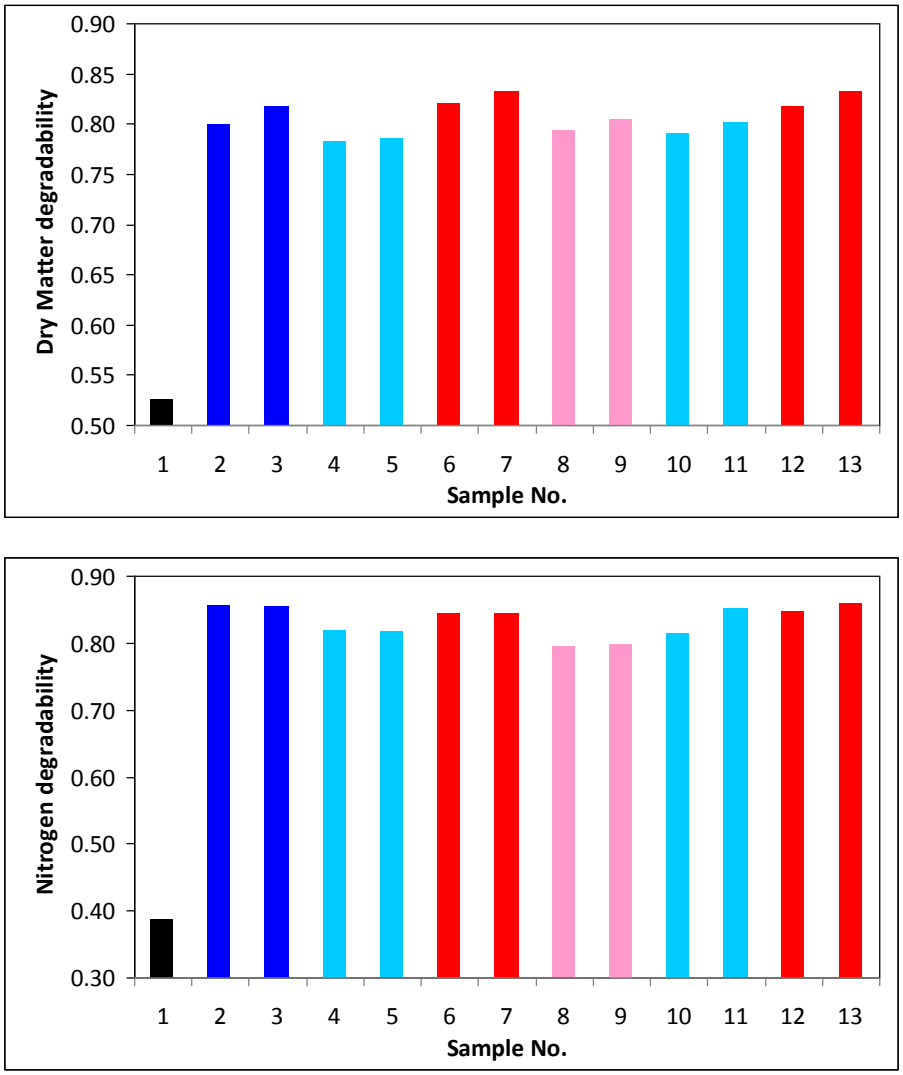


Figure 45 Dry Matter degradability (top) and Nitrogen degradability (bottom) for extruded DDGS.

Sample conditions are specified in Table 71. Sample 1 (black) = raw DDGS. Blue/Light Blue represents 30% moisture, Red/Light Red represents 38% moisture, with the darker colours representing extrusion at 25°C, the lighter colours representing extrusion at 60°C. The left bar in each pair of samples 2-9 is extrusion at 300 rpm, the right bar at 500 rpm. Samples 10-13 represent extrusion through a die or double extrusion, as specified in Table 71.

6.4.5 Enzymatic reduction of syrup viscosity

The scope for enzymatic reduction of syrup viscosity was investigated by applying a range of enzymes to syrup samples supplied by Ensus in December 2012 and March 2013. Table 72 lists the enzymes used for the investigation of the December 2012 sample, and Table 73 lists the enzymes used for the investigation of the March 2013 sample. The first five enzymes were the same in each case, the latter enzymes in each table, in italics, were not used for both investigations.

Table 72 Enzymes investigated for reduction of syrup viscosity, using a syrup samples supplied by Ensus in December 2012, based on a feedstock of 20% Maize and 80% Wheat.

Enzyme Type	Trade Name	Source
Alpha-amylase	Spezyme CL	ADAS
Beta-glucanase	Optimash TBG	ADAS
Beta-glucanase/Xylanase	Optimash BG	ADAS
Protease	Fermgen	ADAS
Saccharifying enzymes	Distillase CS WB	ADAS
Endoprotease	-	*Sigma Aldrich*
Hemicellulase	-	*Sigma Aldrich*

Table 73 Enzymes investigated for reduction of syrup viscosity, using a syrup samples supplied by Ensus in December 2012, based on an unspecified feedstock.

Enzyme Type	Trade Name	Source
Alpha-amylase	Spezyme CL	ADAS
Beta-glucanase	Optimash TBG	ADAS
Beta-glucanase/Xylanase	Optimash BG	ADAS
Protease	Fermgen	ADAS
Saccharifying enzymes	Distillase CS WB	ADAS
Bacterial Xylanase	*Belfeed B*	*Puratos*

Figure 46 shows the effect of enzymes on RVA viscosity at 40°C and 100 rpm of the December 2012 syrup sample. Clearly all of the enzymes were able to reduce the viscosity significantly from its base level of around 580 cP after 5 minutes (shown in black). Whilst recognising that the enzymes were unlikely to be pure and likely to exhibit side activities, nevertheless, this appears to indicate that numerous components contribute to viscosity, including arabinoxylans, beta-glucan, protein and residual starch. The greatest reductions were achieved from mixtures of enzymes – the saccharifying enzyme mixture (Distillase CS WB), and the beta-glucanase/xylanase mix (Optimash BG), the latter achieving the greatest reduction down to about 50 cP, and much greater than the beta-glucanase or the hemicellulase on their own.

The process in December 2012 was using a combination feedstock of 20% Maize and 80% Wheat. By March 2013 the feedstock had changed, information about which was not made available. Figure 47 shows the effect of enzymes on RVA viscosity of the March 2013 syrup sample. In this case the results were very different from those for the December 2012 sample. The base viscosity of the syrup was much higher at around 1500 cP, probably implying a more concentrated syrup. The application of the various enzymes to this syrup had little effect. Once again the saccharifying enzymes and the beta-glucanase/xylanase mix gave the greatest viscosity reductions, down to around 1400 cP. However, the alpha amylase, bacterial xylanase, beta-glucanase and protease all increased the viscosity slightly. This may just reflect experimental error (time constraints prevented replicate analyses), or could indicate the release of viscosity-enhancing molecules as a result of the enzymes attacking the particulate structure of the DDGS. In general, these results

support the argument that despite the use of enzymes during bioethanol processing, significant scope for viscosity reduction can remain in the syrup, dependent on the details of its provenance.

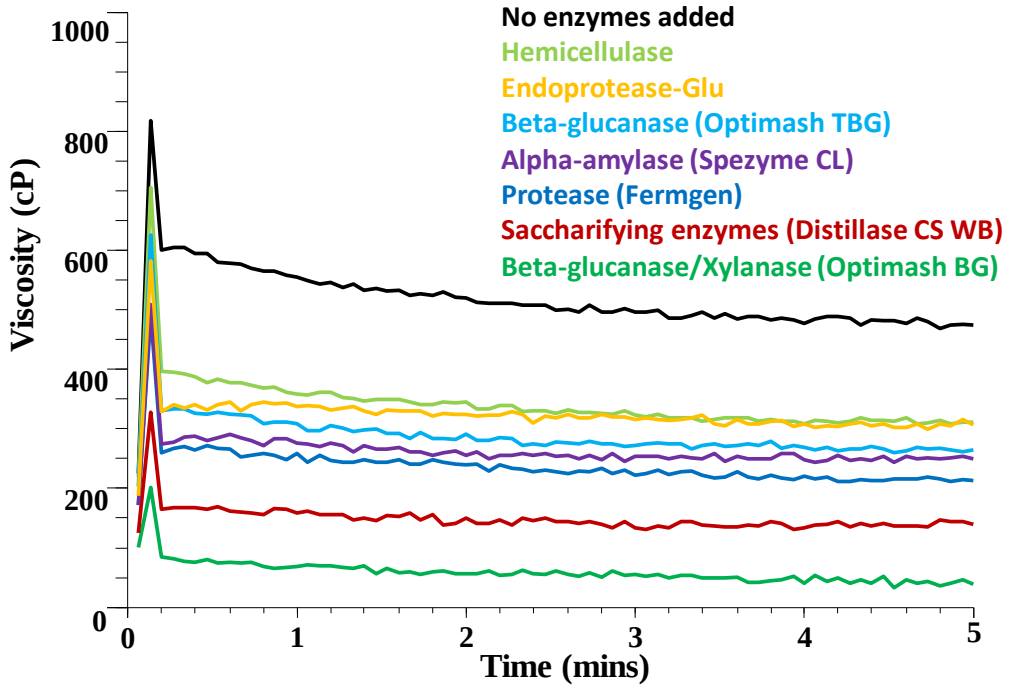


Figure 46 Effect of enzymes on RVA viscosity of the December 2012 syrup sample.

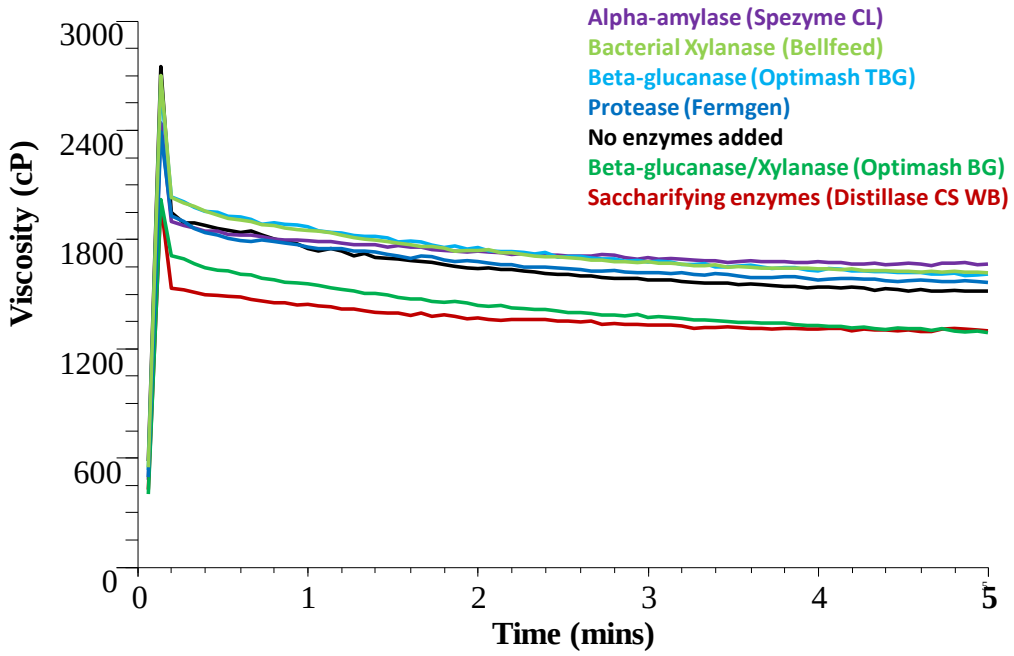


Figure 47 Effect of enzymes on RVA viscosity of the March 2013 syrup sample.

6.5 Discussion

The remit of the Processing Sub-group was to identify and investigate processing options to enhance the nutritional quality of DDGS for use in animal feed. A number of options were identified and investigated at the level of establishing principles; time and resource constraints precluded more detailed investigations of any of the identified options, but all of them were shown to have merit and potential. Whether any one or a combination would actually be commercially viable, in terms of adding sufficient extra value relative to the additional costs of processing, would require a full techno-economic evaluation within the context of a specific plant; the nature of biorefineries makes the economics of processing options very context specific. An emphasis of this report has been on the necessity for integrated processing, and indeed integrated thinking, to be able to evaluate options and deploy them in ways that exploit efficiencies and synergies in order to add value.

The four options identified and investigated were:

1. Arabinoxylan extraction. This would add value to DDGS by reducing its fibre content, making it nutritionally more suited to non-ruminants, while also producing a higher value co-product that could have potential as a food ingredient. The economic opportunity to produce arabinoxylans arises from the context of the bioethanol plant, as the AX production process uses ethanol as a precipitant (Mustafa *et al.*, 2007; Du *et al.*, 2009; Misailidis *et al.*, 2009). The technical challenges to introduce AX extraction commercially include optimising extraction conditions, establishing the functionality of AX for different applications, clarifying suitable sources of AX and extraction conditions for specific functional properties, and characterising AX extracts in order to design extraction processes and understand functionality.

2. Dry fractionation of DDGS. This has the potential to produce fraction enriched in either protein or fibre. On its own this does not add value, as the total available protein remains constant, but if these streams are further processed then the potential to add more targeted value is enhanced; for example, a fibre-rich stream may be more suitable for AX extraction. More generally, the concept of a biorefinery rests on the potential for efficient operation through extensive process integration, arising from a range of co-products and hence a more complex process that gives opportunity for integration. Fractionation underpins this complexity and hence the scope for process integration.

3. Fungal fermentation of DDGS. This has the potential to enhance the amino acid profile of DDGS to make it more suitable for animal nutrition. The extra costs of an additional complex fermentation step, relative to the extra benefit, may make this economically unattractive, but such an evaluation would require a full contextual techno-economic evaluation, based on further studies to optimise the fungal fermentation for the most beneficial amino acid composition.

4. Intense mechanical working. This option would be relatively straightforward to implement via installation of an extruder, if the greater degradability were considered beneficial for particular animal feeds.

Fractionation and conversion are the two keys to an integrated biorefinery co-producing a range of products. The dry fractionation and AX extraction options identified above are examples of fractionation, while the extrusion and fermentation options are examples of conversions – conversions that can be more effectively targeted if applied to specific fractions.

Figure 48 below describes the current process for co-production of bioethanol, DDGS and CO_2, then presents a conceptual process that illustrates how these four additional operations could be introduced into a more complex integrated biorefinery, along with additional enzymic treatment of the syrup to reduce its viscosity. This more complex biorefinery produces AX as an additional co-product, extracted from a high fibre stream produced from dry fractionation of just the dried DDG, and integrated with the ethanol production. The protein-rich stream from this fractionation is recombined with the residue from the AX extraction and extruded to restructure the protein. The protein is then fermented to alter its amino acid composition, before being combined with the enzyme-treated Syrup stream during drying to produce an Enhanced DDGS that has lower AX and hence a higher protein content, better protein quality through fungal fermentation, and more degradable protein through extrusion. It is emphasised that this is an illustrative concept, not a proposal for a viable process, but it serves to illustrate the nature of integrated biorefinery generally and the specific integration of the options identified in the current work for enhancing the nutritional quality of DDGS.

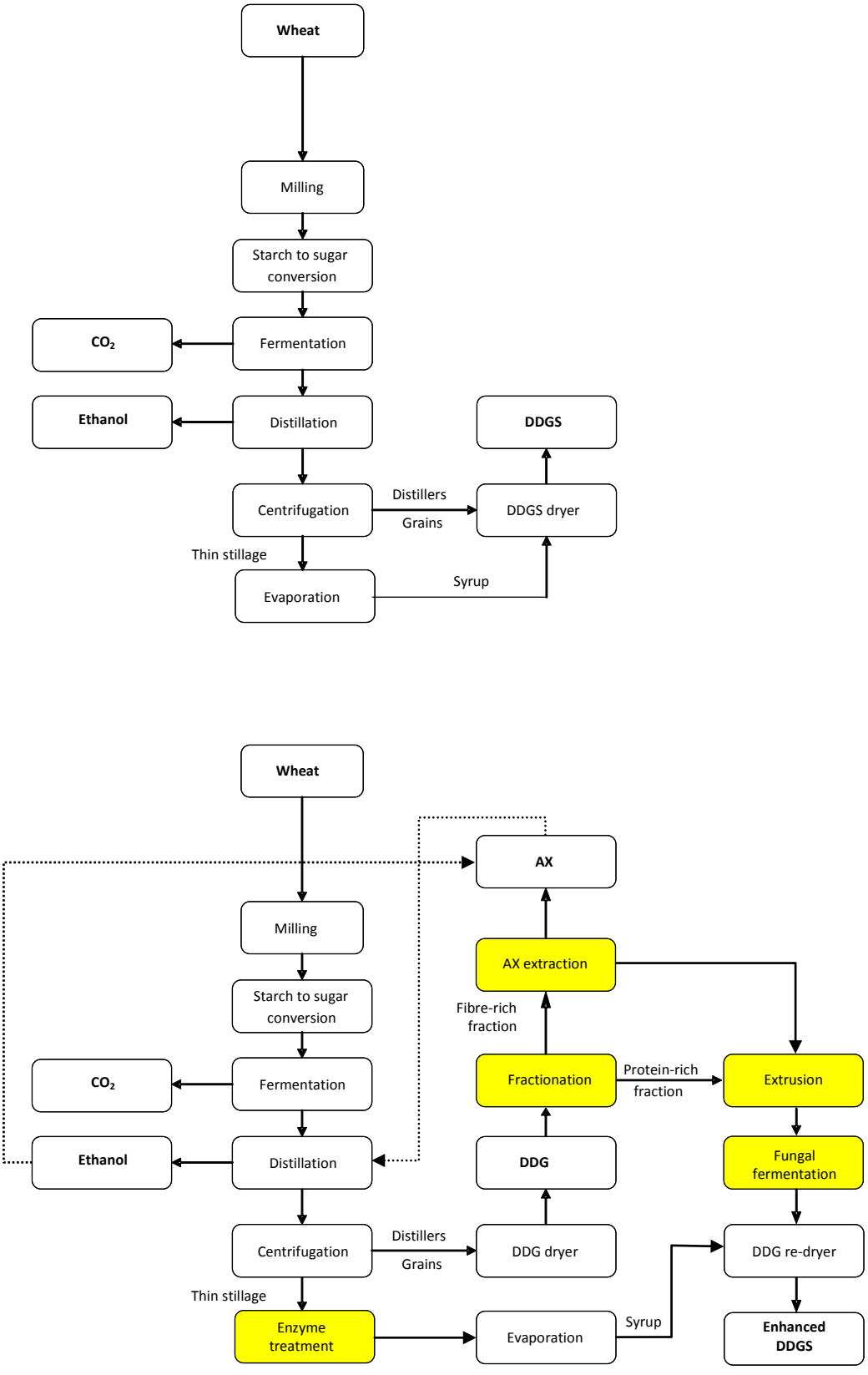


Figure 48 The conventional bioethanol production process with co-production of DDGS and CO_2 (top), and an illustrative concept of an integrated biorefinery employing dry fractionation, AX extraction, extrusion, fungal fermentation and enzyme treatment of the syrup to produce an Enhanced DDGS and an additional Arabinoxylan co-product (bottom).

6.6 Recommendations to industry

The purpose of this subtask within the ENBBIO project was to identify and create options by which industries producing DDGS might enhance the quality and value of their DDGS. A general recommendation is that this will happen most effectively in the context of integrated approaches that allow additional product revenue streams and more efficient operation. The authors recognise the technical, commercial and legislative constraints that dictate and direct the timescale and practicality of adopting some of these approaches. Recognising these, the general message is that a long-term view in which DDGS is produced in increasingly integrated biorefineries will provide helpful guidance and direction for the development of the industry. The opportunity to extract arabinoxylan is particularly promising as a win-win solution that would enhance the nutritional quality and commercial scope of DDGS while producing an additional high value product. Integrated biorefineries provide complexity, which is a precondition to efficient operation; more complex processes also provide scope for enhancing product consistency, a goal not specifically addressed in the current work but of importance to the industry.

7. Land use and environmental benefits of DDGS

7.1 Impacts of bioethanol co-products on global land use and substitution of protein imports for the UK livestock

7.1.1 Background

An important element of the ENBBIO project was to quantify the potential environmental benefits of bioethanol production from wheat, specifically focussing on the value of the co-product and its value in the animal feed supply chain.

Just prior to the start of the ENBBIO project, the production of bioethanol from cereal feedstocks (and other 'food' crops) had been the subject of intense debate between agricultural economists, environmental campaigners and the biofuels industry. The concept of producing ethanol from cereals had moved from a position, in the early 2000's, where it was initially seen as positive both for the environment and the economy, because it offered a way of getting new resources and investment into agriculture, thereby supporting diversification of the farming industry, and secondly, it offered a way of decarbonising transport by introducing ethanol into the liquid fuel supply chain and thereby contributing to meeting the EU's Green House Gas (GHG) emissions reduction targets.

However, in 2008, Searchinger *et al*. published a paper suggesting that the benefits in terms of GHG savings had been overestimated because expansion of biofuels (in the U.S. in their example) would lead to an increase in demand for crop commodities which would result in increased production in areas outside the U.S. If this expansion was achieved by new cropping on virgin land with high carbon stocks, these indirect land use changes (ILUC) would lead to high GHG emissions from conversion to arable land, which could negate or even eliminate the GHG savings from the original biofuel production.

This project cannot begin to answer all of the questions that these arguments pose. However, it has allowed the partners to consider one of the benefits of biofuel production, which is the value of the DDGS co-product.

For Europe, the importance of protein for the animal feed industry cannot be understated. The EU27 import over 15 Mt of soya beans and 23.6 Mt of SBM (equivalent to an original production of ca. 45 Mt of whole soya beans). When other imported proteins are added in, for instance sunflower meal, the scale of the challenge to reduce protein imports for Europe and the UK is clearly seen. While other parts of the world with growing economies compete with us for commodities like SBM, there is a threat to protein supplies for the feed industry. This project therefore aimed to assess the

potential value of wheat DDGS as a substitute for imported proteins like SBM as a means to help guarantee the protein supply for the UK livestock industry.

7.1.2 GHG accounting in bioethanol production and the valuation of DDGS

It should be noted that early GHG accounting methodology for biofuels (and used initially in the UK by the Renewable Fuels Agency) allowed a 'co-product credit' for production of DDGS in general. The values of such co-product credits were estimated using a 'proportional allocation' method using the GHG costs of growing, harvesting, processing transporting and importing, in this case, N American SBM into the EU, coupled with a single substitution ratio for kg SBM substituted per kg wheat DDGS. The derivation of this methodology has been discussed by Punter *et al*. (2004) and Edwards *et al*. (2006). In a European Joint Research Centre (JRC, 2007) report, the proportional allocation of SBM by wheat DDGS was based on a theoretical substitution ratio of 0.78 t SBM/t DDGS. This substitution ratio was in part based on a protein content of 38.5 g/100 g for wheat DDGS which can now be seen from elsewhere in the report as an over estimate of the true CP content, but there are many other deficiencies which could be levelled at this over-simplistic model. However, by 2010 and following the harmonisation of biofuel accounting methodology within the 27 EU member states, the co-product credit based on a protein substitution (proportional allocation) methodology as described above was abandoned. Instead, co-product credits were calculated on a simple 'energy allocation' basis under the protocol of the Renewable Energy Directive (RED; 2009/28/EC). This meant that there was even less of a driver to quantify the nutritional value of DGGS and its broader benefits, as far as livestock production was concerned. Moreover the RED, did not address the more contentious ILUC question, although to some extent this was reviewed both during and following the so-called Gallagher review in the UK (Gallagher, 2008).

7.1.3 Previous estimates of the value of DDGS

Weightman *et al*. (2010) considered the supply of ethanol co-products in Europe, that is wheat DDGS and sugar beet pulp, and estimated the effects on land usage, and displacement of other feed ingredients when these co-products are used. The paper by Weightman *et al*. was prepared before there was significant wheat bioethanol production taking place in the UK (i.e. neither Ensus or Vivergo were in production), and hence the typical inclusion levels and substitution ratios (e.g. kg soya bean meal displaced per kg wheat DDGS) were those prevalent at the time. Nevertheless, different values were used for the different livestock species, unlike the common figure used by JRC (2007), and were guided by modelling specific economic scenarios based on a typical UK least cost ration formulation system, using wheat DDGS to substitute SBM plus wheat and other cereal by-products. These values are shown in Table 74 (Scenario A)

In the 2009 scenario (A), the relative proportions of each livestock class were based on the production volumes of compound feeds per livestock type within the EU. Clearly this is a

simplification as there are many ruminant livestock fed on pasture and conserved forage, as well as straights fed on farm, as acknowledged by Hazzledine *et al*.(2011) (see following section) but gives a pragmatic starting point for the analysis.

The inclusion levels and substitution ratios were given for both existing (2010) usage and future high usage scenario (B) proposed by Lywood *et al*. (2009). In part the conservative values shown for the 2009 scenario A were based on the fact that while it would be possible to force more wheat DDGS into the diet to simulate the effect of flooding the market with DDGS, it was noted that the effect at the diet level would be to reduce the SBM/wheat DDGS substitution ratio. This is because feed materials other than SBM would be forced out of the diet e.g. barley and other mid-protein feeds like RSM.

Table 74 Inclusion levels and substitution ratios of SBM with wheat DDGS from various sources, estimated prior to and at the start of the ENBBIO project.

Scenario/ Livestock type	Relative proportion fed to each livestock type (poultry=1)	Typical inclusion limits of wheat DDGS (g/kg)	Substitution (t SBM/t DDGS)
A) 2009 EU scenario (Weightman et al., 2010)			
Pig	2.05	61	0.35
Poultry	1.00	65	0.56
Ruminant	4.34	45	0.26
Average			0.33
B) Future high usage scenario (Lywood et al., 2009)			
Pig	1.11	150	0.59
Poultry	1.00	100	0.58
Ruminant	0.81	400	0.62
Average			0.60
C) 2011 GB moderate usage (1.0 Mt wheat DDGS) scenario (Hazzledine et al., 2011)			
Pig	0.70	12	NA
Poultry	1.00	7	NA
Ruminant	21.56	196	NA
Average			0.24
D) 2011 GB high usage (1.63 Mt wheat DDGS) scenario (Hazzledine et al., 2011)			
Pig	1.00	136	0.25
Poultry	1.00	56	0.37
Ruminant	2.83	202	0.28
Average			0.29

NA, Data not available from paper

7.1.4 Modelling GB feed supply

Another feature of the high usage scenario (B; Table 74) was that Lywood *et al*. (2009) envisaged the greater proportion of the DDGS would ultimately go into pig and poultry, rather than ruminant diets. This could be questioned, as traditionally ruminant diets have been the main user of distillers

grains in their various forms, but the point was made that SBM shows greatest nutritional value in non-ruminant diets and hence its replacement by wheat DDGS was the bigger prize.

Nevertheless, it should be noted that when Hazzledine *et al*. (2011) modelled GB feed supply, they supported the traditional view that the greatest value of wheat DDGS is in ruminant diets (Scenario C; Table 74).

The Hazzledine 'GB' model, with a moderate DDGS availability of 0.77 Mt of wheat DDGS resulted in all but 2 kt being used in the ruminant sector. Wheat DDGS mainly replaced soya meal (0.33 replacement), sunflower meal (0.30) and rape meal (0.22). Additional barley was used (0.24) and less wheat (0.33). When the total DDGS available was increased to 1 Mt (Scenario C; Table 74) some 30 kt were used in pig and 43 kt in poultry. Wheat DDGS again replaced largely soya meal (0.24), sunflower meal (0.21) and rape.

In the Hazzledine model, with a high DDGS availability (Scenario D; Table 74) of 1.62 Mt (12.4% of total feed) approximately 0.95 Mt (59%) was predicted to be used in ruminant feeds with the remainder equally utilised in pig and poultry feeds, although there were some uncertainties as the authors noted that it was impossible to find data on the amount of straights fed to ruminants on farm. Soya bean meal replacement rate was 0.29 (pig 0.25, poultry 0.37, ruminant 0.28) and cereal 0.28. Other commodities replaced by wheat DDGS included extracted sunflower meal (0.18), maize gluten feed (0.13) and palm kernel extractions (0.12).

A comparison of the three models shown in Table 74 highlights many of the uncertainties associated with usage of DDGS. The most striking differences between the various scenarios are;

1. The relatively small proportion of DDGS predicted to go into ruminant feeds (0.81) relative to poultry in Lywood *et al*. (2009), compared to 2.83 by Hazzledine *et al*. (2011) in their high usage model.
2. The low inclusion rate of 45 g/kg used for ruminants by Weightman *et al.* 2010, compared to the value of 400 g/kg by Lywood *et al.* (2009). The 45 g/kg was based on the actual output of an LCRF exercise using a basket of feeds based on October 2009 prices, while the 400 g/kg was based on the maximum inclusion limit recommended at the time (e.g. see Cottrill *et al*., 2007),
3. The high values for average substitution ratios (0.6 SBM/wheat DDGS) by Lywood *et al*. (2009) compared to the other two scenarios (ca. 0.3).

7.1.5 Analysis using updated substitution ratios (outputs of ENBBIO project)

The research carried out in the ENBBIO project aimed to: quantify sources of variability in wheat distillers grains and solubles (W- DDGS); identify opportunities to enhance their value; consider innovative processes to reduce fibre content (for non-ruminants); and to quantify the contribution of the co-products to the overall GHG balance of UK crop, livestock and ethanol production. The work carried out under the ENBBIO project allows us to revisit the original evaluations with updated figures, and in which the industry can have more confidence. Table 75 shows the maximum inclusion limits taking into account the work carried out in the ENBBIO project, and/or the likely inclusion limits based on the experience of the ENBBIO commercial partners.

To give an idea of the potential amount of wheat DDGS that could be used in UK feed, the data from Table 75 have been combined with statistical data on livestock feed manufactured (Table 76). If the typical inclusion levels started in Table 75 were simply applied to 16.1 Mt of feed, this would represent usage of 1.97 Mt of wheat DDGS. This is in excess of the total amounts of wheat DDGS which will produced when Ensus and Vivergo are running at full capacity (350 and 500 kt of wheat DDGS respectively). Even adding in 280 kt of mixed w/bDDGS from the Scotch whisky industry, only brings the total to ca. 1.3 Mt of DDGS. This is closer to the volume of 1 Mt wheat DDGS used in the GB moderate usage Scenario C in Table 74. Therefore there are two realistic scenarios which can be used to show the likely range in usage from Table 74: Scenario C, practically all wheat DDGS in ruminant feeds, or; D, split more evenly between ruminant and non ruminant livestock types Table 77.

Table 75 Inclusion rates for wheat DDGS in diets, by animal type based on consensus views of ENBBIO consortium.

	Inclusion levels (g/kg)			
	Maximum	Future Typical	In trials	Reference*
Pigs †				
Growers	200	100**	0 – 300kg	UoN & Harper trial
Finisher	300	100**	0 – 300kg	UoN & Harper trial
Poultry				
Layers	100	75**	75	Noble Foods trial
Broiler	100	100**	100	H2S 2nd study
Ruminants				
Dairy cows	350	200	210	UoN trial
Beef			170	Commercial trial
Beef/heifers	300	150		Pers. comm
Calves	200	150		Ibid
Ewes	350	200		Ibid
Lambs	150	100		Ibid
Milk sheep/goats	300	200		Ibid

*, If no specific trial data, then personal communication is by commercial members of ENBBIO consortium.

†, Pig data are based on trials using pellets, not meal.

**, Typical inclusion in least cost formulation using 2014/15 raw material prices.

In reality, these will be affected by the availability and raw material price of other commodities both imported and home-grown, like rape seed meal (RSM), but give two pragmatic scenarios. The volumes of wheat DDGS used and the amount of SBM which could be substituted are shown in Table 77. Various combinations of the ratios of ruminant to non ruminant feed to match Scenarios C and D (Table 74) while keeping the total volumes of feed below the actual levels and utilising ca. 1 Mt of wheat DDGS were used to generate four scenarios E-H. The amount of SBM which is likely to be substituted varies between 287 and 335 kt, or approximately 11.1-13.4 % of the UK's SBM annual imports of ca. 2.5 Mt.

Table 76 UK Feed stats Oct 2013 to September 2014. Source: Defra and DARDNI

Livestock/diet type	UK feed ktonnes	Livestock /diet type	UK feed ktonnes
Cattle		*Pigs*	
All calf feed	243	Pig starter and creep feed	77
Dairy compound	2,604	Link / early grower feed	158.5
Dairy blend	1,044	Pig growing feed	853.1
Other cattle compound	770	Pig finishing feed	1615.4
Other cattle blend	644	Pig breeding feed	595.4
Protein concs	85	Protein concentrates	13
Total cattle	*5,390*	*Total pig*	*3,352*
Sheep		*Poultry*	
Breeding sheep compound	331	Chick rearing feed	158
Breeding sheep blend	48	Layer feed (incl. integrators)	1,383
Growing and finishing compound	337	Broiler chicken feed (incl. integrators)	3,783
Growing and finishing blend	58	Poultry breeding and rearing feed (incl. integrators)	419
Protein concs	5	Turkey feed (incl. integrators)	574
		All other poultry feed	284
		Protein concentrates	2
Total sheep	*780*	*Total poultry*	*6,600*
Grand total	*16,122*		

Table 77 Predicted quantity of SBM which could be substituted in UK livestock diets through utilisation of up to 1.2 Mt wheat DDGS, using various scenarios based on different feed volumes, inclusion limits and common substitution ratios.

Scenario	Total feed (kt)	Inclusion level (g/kg)	DDGS (kt)	SBM substitution ratio	SBM substituted (kt)
E) Feed allocations & inclusion levels based on scenario C, Table 74*)*					
Pigs	197	12	2	0.25	1
Poultry	282	7	2	0.37	1
Cattle	5304	196	1,040	0.28	291
Sheep	774	196	152	0.28	42
Total	*6558*		*1,196*		*335*
F) Feed allocations & inclusion levels based on scenario D, Table 74*)*					
Pigs	1,500	136	204	0.25	51
Poultry	1,500	56	84	0.37	31
Cattle	3,704	202	748	0.28	210
Sheep	541	196	106	0.28	30
Total	*7,245*		*1,142*		*321*
G) As scenario E, but with inclusion levels based on consensus in Table 75					
Pigs	197	100	20	0.25	5
Poultry	282	75	21	0.37	8
Cattle	5,304	175	928	0.28	260
Sheep	774	175	135	0.28	38
Total	*6,558*		*1,105*		*311*
H) As scenario F, but with inclusion levels based on consensus in Table 75					
Pigs	1,500	100	150	0.25	38
Poultry	1,500	75	113	0.37	42
Cattle	3,704	175	648	0.28	182
Sheep	541	175	95	0.28	26
Total	*7245*		*1,005*		*287*

7.1.6 Other benefits of using DDGS

When DDGS is used in animal feeds it will displace some other ingredients, as there is less demand for them. It could be considered in very simplistic terms, that taking one tonne of wheat for bioethanol removes one tonne of wheat from the food supply chain, but this is an over simplification since most of the wheat in the UK is used for animal feed, and wheat DDGS returns the protein to the animal feed industry thus replacing any protein from feed wheat (but not the energy) and/or wheat milling by-products such as wheat 'middlings' and bran fractions from the human food industry. Moreover it could displace other ingredients like SBM which require land in other parts of the world.

Assuming a conversion of wheat to wheat DDGS of 0.33, and producing ca. 1 Mt of DDGS, then the wheat bioethanol industry plus other suppliers of wheat DDGS like the Scotch Whisky industry, is likely to consume in the order of 3 Mt of wheat per annum. With an average UK wheat yield of 7.48 t/ha (average yields 2009-2013; FAO stats) this relates to a UK production area of 405 kha out of a total harvested wheat area of 1.68 Mha (24% of UK wheat area).

As noted above, the wheat DDGS equivalent from this land area could substitute between 287 and 335 kt of SBM. Assuming a conversion from whole soya beans to SBM of 0.8 (Weightman *et al*., 2010) this equates to 359 – 419 kt whole soya. With an average soya bean yield of 2.60 t/Ha (2009-2013 average for the US, Argentina, Brazil, Paraguay and Uraguay) this relates to ca. 150 kha of land for soya production at the mid point in the estimates.

In addition, other commodities are substituted by wheat DDGS as noted by Hazzledine *et al.* (2011), e.g. sunflower meal (SFM) may also be displaced from livestock feeds. With a substitution ratio of 0.33 (see Table 8 in Hazzledine *et al*., 2011) this would equate to 330 kt of SFM per annum, equivalent to 508 kt sunflower seed (assuming 35% oil content). Of the three major producers of sunflowers, Argentina, France and Ukraine, the latter dominates production with 2.96, 1.68 and 8.25 Mt per annum (sunflower seed) respectively. With an average yield (2009-2013) of 1.73 t/ha for sunflower seed, this would equate to 299 kha of sunflower production in Ukraine.
Finally, some 130 kt of wheat would be displaced by wheat DDGS (substitution ratio 0.13 from Table 8, Hazzledine *et al*., 2011). With an average UK wheat yield of 7.48 t/ha, this equates to 17,426 ha of land in the UK. Thus the 'net' area of land in the UK used for wheat biofuel production would be 388 kha or 23% of UK wheat area.

7.1.7 Summary

The wheat bioethanol industry plus other suppliers of wheat are likely to consume the order of 3 Mt of wheat per annum, grown on 405 kha of UK arable land to produce wheat DDGS, in addition to the bioethanol produced, would potentially substitute for ca. 1Mt of three major commodities used

in animal feeds. This represents existing crop production taking place on 466 kha of land worldwide as summarised below (Table 78). Whether there is a net benefit to the UK depends on a UK wheat surplus, which was present annually in the mid 2000's when the development of the bioethanol industry was planned.

Table 78. Summary of key crop commodities substituted and their respective production volumes and areas, when ca. 1Mt wheat DDGS (originating from wheat grown on 405 kha of arable land) is used in UK animal feed.

Commodity	Crop and country of origin	Production of crop (kt)	Land area spared (kha)
SBM	Soya beans (N and S America)	389	150
SFM	Sunflower seed (Ukraine)	508	299
Wheat	Wheat (UK)	130	17
Total		*1,027*	*466*

While these estimates of the potential of DDGS to substitute for other protein commodities, are open to debate, we have presented more realistic scenarios e.g. substitution ratios 0.2 – 0.3, compared to those in the earlier literature (0.6 – 0.78: JRC, 2008; Lywood *et al.*, 2009). The estimates here, coupled with the fact that feed producers in the UK will have more confidence in the inclusion of wheat DDGS in ruminant and non ruminant feeds as a result of the project, means that wheat DDGS as a co-product of the bioethanol industries could make a definite contribution to security of protein supplies in the UK.

8. Conclusions

An important element of the ENBBIO project was to quantify the potential environmental benefits of bioethanol production, focussing on the utilisation of the co-products and their value in the animal feed supply chain. Using 3 Mt of wheat grown on 405 kha of UK arable land to produce DDGS, in addition to the bioethanol produced, would potentially substitute for ca. 1Mt of three major commodities used in animal feeds i.e. SBM, SFM and wheat. This represents existing crop production taking place on 466 kha of land worldwide. The extent to which of DDGS will substitute for other commodities, particularly plant proteins, will inevitably show some variation over time, for instance as economic scenarios and the relative prices of different feed ingredients change. However, more realistic scenarios for substitution ratios of DDGS for soya bean meal have been estimated as a result of the project (0.2 – 0.3), compared to those assumed in earlier biofuel GHG accounting methodologies (0.6 – 0.78). An estimated 389 kt of SBM will be substituted, which equates to 150 kha land area spared. There could be additional benefits for the UK from use of wDDGS not quantified here. These estimates, coupled with the fact that feed producers in the UK now have more confidence in the inclusion of wDDGS in ruminant and non ruminant feeds as a result of the project, means that DDGS as a co-product of the bioethanol industries could make a definite contribution to security of plant protein supplies for animal feed in the UK.

The non-ruminant programme was designed to examine the nutritional value of wheat distillers dark grains with solubles (W-DDGS) in poultry and pigs. Nine separate trials were undertaken based on a range of objectives / methodologies. When performance was assessed over the entire first broiler trial, birds fed 5% W-DDGS in the starter experienced an inferior FCR overall with increasing W-DDGS in the grower. Although differences were not, generally, statistically significant, numerical changes between treatments would be of some considerable importance in a production context. A reduction in the coefficient of apparent ileal nitrogen and amino acid digestibility was observed with increasing levels of W-DDGS. The next trial (Nottingham) reported values for apparent ileal digestibility (AID) and standard ileal digestibility (SID) of amino acids were similar to those reported elsewhere in the literature. A large-scale commercial trial (H2S) revealed that there were no differences in liveweight, but better Feed Conversion Ratio with W-DDGS although these diets were more expensive as a result of having to include higher levels of pure amino acids; however cost /kg gain was lower and Production Efficiency Factor (PEF) higher. The trial has shown that the addition of up to 10% W-DDGS into a balanced broiler diet, had no detrimental effects on the technical performance of the birds. The concerns of the effects that W-DDGS may have on litter quality were not shown in the trial work. An initial layer trial (Nottingham) reported that including W-DDGS at up to 18% in diets that were isoenergetic and balanced for digestible amino acids had no effect on performance and egg shell quality; there were no effects of treatment on gut environment / microflora. The next commercial layer trial (Noble) reported that, with an inclusion of 7.5% W-DDGS with the nutritional matrix values ascribed to the raw material in

the formulations by Premier Nutrition, there was no practical difference between the trial and control flocks. In addition and in particular concerns over potential increased seconds from using W-DDGS were not realised. W-DDGS therefore can be safely used in layer diets, in part substituting for imported soya. Whether it is actually used or not will depend on the relative values of the product and other raw materials used in least cost formulated layer diets. However, at recent market values it would not feature in a typical layer diet.

The initial pig trial (Nottingham) examined amino acid digestibility.
An experiment was conducted (Illinois, USA) to determine the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of amino acids (AA) by growing pigs fitted with T-cannulae in five different sources of DDGS from Europe. Using data from the Illinois trial, diets were formulated to be iso-energetic and balanced for SID amino acids in a preliminary growth trial (Nottingham). Growing / finishing pigs are able to tolerate levels of W-DDGS up to 300g/kg in pelleted balanced diets in terms of performance and carcass quality without a significant reduction in performance. In a final commercial growth trial (Tulip, Harper Adams), the inclusion of Wheat Dried Distillers Grains (W-DDGS) at any of the levels in the pelleted diets did not have any negative effects for on farm performance, slaughter characteristics or meat quality. The only significant relationship within the dose response range and structure was FCR in the first two periods with a linear response. The highest inclusion at 30% showed best performance in a number of areas including daily liveweight gain, FCR and slaughter weight. It can therefore be concluded that feeding pigs during the growing and finishing stages with up to 30% W-DDGS included in the diets is an acceptable level.

The ENBBIO ruminant studies achieved their primary objective, which was to evaluate Wheat DDGS (wDDGS) from UK bioethanol production in terms of nutritional value and animal responses to inclusion in typical ruminant diets. The first dairy trial gave an apparent limitation of wDDGS inclusion of ~20% of diet dry matter. Results of sheep ME trials provided evidence to support the hypothesis that the wDDGS used in the first dairy trial had a lower ME content than was assumed during diet formulation. The ME value used for diet formulation was 13.7 MJ/kg DM and the value measured in the sheep ME trial was 12.1 MJ/kg DM. Results of rumen studies showed that degradability characteristics of DDGS vary markedly between sources, probably as a result of proportion of solubles added and heat treatment during drying. Digestibility studies confirmed that there was no significant effect of wDDGS inclusion level on dry matter digestibility. A second dairy trial re-examined the effect of inclusion level of wDDGS. For this trial, diets were formulated with ME values and degradation characteristics determined in advance for the actual batch of wDDGS to be tested. There was no effect of wDDGS inclusion level on intake or performance. In a survey of wDDGS use on commercial beef farms, inclusion levels of 12.5% and 30% of the diet supported good performance levels.

Another element of work within the ENBBIO project was to identify and create options by which industries producing DDGS might enhance the quality and value of their DDGS. A general recommendation is that this will happen most effectively in the context of integrated approaches that allow additional product revenue streams and more efficient operation. There are technical, commercial and legislative constraints that dictate and direct the timescale and practicality of adopting some of these approaches. Recognising these, the general message is that a long-term view in which DDGS is produced in increasingly integrated biorefineries will provide helpful guidance and direction for the development of the industry. The opportunity to extract arabinoxylan is particularly promising as a win-win solution that would enhance the nutritional quality and commercial scope of DDGS while producing an additional high value product. Integrated biorefineries provide complexity, which is a precondition to efficient operation; more complex processes also provide scope for enhancing product consistency, a goal not specifically addressed in the current work but of importance to the industry.

At the outset of the ENBBIO project, the animal feed industry had concerns relating to the risk of mycotoxins in DDGS. This was because the non-starch and non-sugar components are concentrated three times in DDGS, and so, mycotoxins are theoretically also concentrated three times in the non-fermentable residue. However in a large scale bioethanol refinery, dilution with other grain not contaminated with mycotoxin on a regular basis, means the risk of significant levels in the DDGS is very low. Nevertheless the animal feed industry still seeks to minimise the risk of mycotoxin contamination where possible. The risk of mycotoxin contamination appears to be lower than the industry may have initially thought, although further research would be warranted to investigate this further. Plant breeders are working towards fusarium resistant wheats which, coupled with high starch content, would make ideal wheats for bioethanol. On a large scale, with mixing of large volumes of wheat, individual batches of wheat will tend to smooth out variations in DDGS quality, but improvements through plant breeding can help move the wheat supply chain as a whole in the direction of improved feedstock quality.

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Appendix 1. Diet formulations and assumed composition

Starter 0-14 days

	Formulated	Formulated
Ingredient	0 DDGS	5% DDGS
Wheat - Feed	57.25%	55.11%
DDGS	0.00%	5.00%
Soybean meal 48	36.00%	32.84%
Soy oil	3.43%	3.73%
Salt	0.33%	0.27%
DL Methionine	0.31%	0.31%
Lysine HCl	0.21%	0.29%
Threonine	0.05%	0.06%
Limestone	1.26%	1.29%
Mono Dical Phos	0.74%	0.68%
Quantum 2500	0.02%	0.02%
Vitamin premix	0.40%	0.40%
Crude protein %	24.13	24.09
Poult ME MJ/kg	12.54	12.54
Poult ME kcal/kg	3000	3000
Calcium %	0.95	0.95
Phos %	0.76	0.76
Avail Phos %	0.45	0.45
Fat %	4.92	5.50
Crude Fibre %	2.63	2.87
ADF %	3.02	3.94
NDF %	7.57	9.09
Met %	0.65	0.65
Cys %	0.40	0.40
Me+Cys %	1.07	1.07
Lys %	1.43	1.43
His %	0.59	0.59
Tryp %	0.30	0.29
Thr %	0.94	0.94
Arg %	1.56	1.50
Iso %	0.99	0.98
Leu %	1.76	1.74
Phe %	1.12	1.12
Tyr %	0.80	0.79
Val %	1.07	1.07
Gly %	0.97	0.97
Ser %	1.12	1.12
Phe+Tyr %	1.92	1.90
D Met%	0.59	0.59
D Cys%	0.36	0.36
D Me+Cys %	0.96	0.96
D Lys %	1.29	1.29

D His %	0.53	0.53
D Tryp %	0.27	0.27
D Thr %	0.85	0.85
D Arg %	1.40	1.35
D Iso %	0.89	0.88
D Leu %	1.58	1.56
D Val %	0.96	0.96
D Gly %	0.87	0.88
D Ser %	1.01	1.01
Phytate P %	0.24	0.23
Na %	0.16	0.16
Cl %	0.29	0.27
K %	0.99	0.97
Linoleic acid %	2.32	2.55
Na+K-Cl	241.72	241.57
DUA	413.48	420.08
Sulphur%	0.24	0.23
Magnesium	0.16	0.17
Betaine	0.72	0.70
Choline	1441.10	1437.79
Copper	17.69	17.73

Grower phase 15-28 days

	Formulated	Blended	Blended	Formulated
	0% DDGS	6% DDGS	12% DDGS	18% DDGS
Proportion of control		0.66	0.33	
Wheat - Feed	61.58%	58.37%	55.26%	52.15%
DDGS	0.00%	6.12%	12.06%	18.00%
Soybean meal 48	30.29%	27.00%	23.80%	20.60%
Soy oil	5.36%	5.78%	6.18%	6.58%
Salt	0.33%	0.25%	0.18%	0.10%
DL Methionine	0.25%	0.25%	0.24%	0.24%
Lysine HCl	0.17%	0.25%	0.33%	0.40%
Threonine	0.03%	0.04%	0.04%	0.04%
Limestone	1.18%	1.22%	1.26%	1.30%
Mono Dical Phos	0.38%	0.31%	0.23%	0.16%
Quantum 2500	0.02%	0.02%	0.02%	0.02%
Vitamin premix	0.40%	0.40%	0.40%	0.40%
Crude protein %	21.83	21.98	22.12	22.27
Poult ME MJ/kg	13.17	13.17	13.17	13.17
Poult ME kcal/kg	3150	3150	3150	3150
Calcium %	0.85	0.85	0.85	0.85
Phos %	0.67	0.66	0.65	0.64
Avail Phos %	0.37	0.37	0.37	0.37
Fat %	6.62	7.36	8.08	8.80
Crude Fibre %	2.54	2.84	3.13	3.42
ADF %	2.87	4.01	5.12	6.22
NDF %	7.51	9.37	11.17	12.98
Met %	0.56	0.56	0.55	0.55
Cys %	0.37	0.37	0.38	0.38
Me+Cys %	0.95	0.95	0.95	0.95
Lys %	1.24	1.24	1.24	1.24
His %	0.53	0.53	0.54	0.54
Tryp %	0.27	0.27	0.26	0.26
Thr %	0.83	0.83	0.83	0.83
Arg %	1.38	1.32	1.26	1.20
Iso %	0.88	0.88	0.88	0.88
Leu %	1.57	1.56	1.56	1.55
Phe %	1.01	1.01	1.01	1.02
Tyr %	0.71	0.71	0.70	0.70
Val %	0.96	0.97	0.98	0.99
Gly %	0.88	0.89	0.90	0.91
Ser %	1.01	1.02	1.03	1.04
Phe+Tyr %	1.72	1.72	1.72	1.72
D Met%	0.51	0.50	0.50	0.49
D Cys%	0.33	0.34	0.34	0.34

D Me+Cys %	0.85	0.85	0.85	0.85
D Lys %	1.12	1.12	1.12	1.12
D His %	0.48	0.48	0.48	0.48
D Tryp %	0.24	0.24	0.24	0.24
D Thr %	0.75	0.75	0.75	0.75
D Arg %	1.24	1.19	1.13	1.08
D Iso %	0.79	0.79	0.79	0.79
D Leu %	1.41	1.41	1.40	1.39
D Val %	0.86	0.87	0.88	0.89
D Gly %	0.79	0.80	0.81	0.82
D Ser %	0.91	0.92	0.93	0.94
Phytate P %	0.22	0.22	0.22	0.21
Na %	0.16	0.16	0.16	0.16
Cl %	0.28	0.26	0.24	0.21
K %	0.89	0.87	0.86	0.85
Linoleic acid %	2.76	3.01	3.25	3.49
Na+K-Cl	216.68	219.95	223.12	226.29
DUA	392.51	403.74	414.64	425.55
Sulphur%	0.21	0.21	0.21	0.21
Magnesium	0.15	0.16	0.17	0.18
Betaine	0.78	0.74	0.71	0.68
Choline	1319.94	1326.70	1333.26	1339.82
Copper	17.01	17.25	17.49	17.73

Appendix 2: Diets

Grower

	G1	G2	G3	G4
Barley	300.0	234.7	169.3	104.0
Wheat	357.0	387.7	418.5	449.2
Wheatfeed	125.0	83.3	41.7	-
Soya Hipro Ext	132.8	103.4	73.9	44.5
Rapeseed Ext	50.0	50.0	50.0	50.0
Premix	2.5	2.5	2.5	2.5
L-Lysine	6.0	7.8	9.6	11.5
DL_Methionine	0.6	0.6	0.5	0.5
Threonine	1.3	1.4	1.6	1.7
L-Tryptophan	0.0	0.1	0.1	0.2
Vitamin E	0.2	0.2	0.2	0.2
Finase	0.1	0.1	0.1	0.1
Limestone	9.3	10.1	10.8	11.5
DiCalcium phosphate	2.8	1.9	0.9	-
Salt	3.8	3.6	3.5	3.3
Sodium bicarbonate	3.5	2.3	1.2	0.0
Soya Oil	5.0	5.0	5.0	5.0
Fat	-	5.3	10.6	15.9
W-DDGS	-	100.0	200.0	300.0
TOTAL	1000.0	1000.0	1000.0	1000.0

Grower: Diet composition (g/kg unless otherwise stated)

OIL B	34.5	43.2	51.8	60.5
PROTEIN	169.2	182.2	195.1	208.1
FIBRE	44.7	47.1	49.5	51.9
ASH	49.4	48.4	47.5	46.5
NaCl	5.0	5.0	5.0	5.0
Ca	6.3	6.3	6.3	6.3
Dig Ca	7.5	7.5	7.5	7.5
P	4.8	4.6	4.4	4.2
Dig P	2.8	2.8	2.8	2.8
Na	2.5	2.6	2.6	2.6
Cu (ppm)	20.6	20.9	21.1	21.4
NDF	139.9	152.3	164.8	177.3
STARCH	399.5	376.5	353.5	330.5
SUGARS	38.5	35.9	33.3	30.8
EB (mEq/kg)	225	220	216	211
SID LYS	9.5	9.5	9.5	9.5
LYS	10.6	10.8	11.0	11.2
MET	3.2	3.3	3.4	3.4
CYS	3.2	3.4	3.6	3.8
THR	7.1	7.4	7.6	7.8
TRP	2.1	2.2	2.3	2.4
HIS	4.1	4.2	4.2	4.2
ILE	6.4	6.6	6.8	7.1
LEU	11.6	12.2	12.7	13.3
PHE	7.7	8.2	8.6	9.1
TYR	5.3	5.6	5.8	6.1
VAL	7.7	8.1	8.4	8.8
NE Pig (MJ/kg)	8.95	8.95	8.95	8.95
SMET:SLYS	3.1	3.0	3.0	3.0
SM+C:SLYS	5.9	6.0	6.0	6.1
STHR:SLYS	6.5	6.5	6.5	6.5
STRP:SLYS	1.9	1.9	1.9	1.9
SHIS:SLYS	3.9	3.8	3.7	3.6
SILE:SLYS	5.9	5.9	6.0	6.0
SLEU:SLYS	10.6	10.8	11.1	11.3
SVAL:SLYS	7.0	7.0	7.0	7.0
SP+T:SLYS	12.2	12.6	13.1	13.5

Finisher

	F1	F2	F3	F4
Barley	300.0	274.5	248.9	223.4
Wheat	345.9	358.1	370.4	382.6
Wheatfeed	175.0	116.7	58.3	-
Soya Hipro Ext	126.4	92.9	59.5	26.0
Rapeseed	25.0	25.0	25.0	25.0
Pig	2.5	2.5	2.5	2.5
L-Lysine	1.7	3.7	5.7	7.8
Threonine	-	0.1	0.3	0.4
Vitamin	0.2	0.2	0.2	0.2
Finase	0.1	0.1	0.1	0.1
Limestone	10.1	10.7	11.4	12.1
DiCalcium phosphate	2.4	1.6	0.8	-
Salt	4.3	4.0	3.6	3.2
Sodium Bicarbonate	1.5	1.0	0.6	0.1
Fat	5.0	8.9	12.8	16.7
W- DDGS		100.0	200.0	300.0
TOTAL	1000.0	1000.0	1000.0	1000.0

Finisher: Diet composition (g/kg unless otherwise stated)

OIL B	35.4	42.5	49.5	56.6
PROTEIN	160.3	171.0	181.7	192.4
FIBRE	45.2	47.5	49.7	51.9
ASH	48.7	47.7	46.6	45.6
NaCl	5.5	5.3	5.2	5.0
Ca	6.3	6.3	6.3	6.3
Dig Ca	7.5	7.5	7.5	7.5
P	4.7	4.5	4.2	4.0
Dig P	2.7	2.7	2.7	2.7
NaCl	2.2	2.3	2.5	2.6
Cu (ppm)	20.8	21.0	21.1	21.3
NDF	149.2	160.1	171.0	182.0
STARCH	403.5	385.3	367.1	348.8
SUGARS	38.0	34.6	31.1	27.6
EB (mEq/kg)	200	199	198	197
SID LYS	7.0	7.0	7.0	7.0
LYS	8.0	8.2	8.4	8.6
MET	2.5	2.6	2.7	2.7
CYS	3.1	3.3	3.4	3.6
THR	5.6	5.8	5.9	6.1
TRP	2.0	2.0	2.0	2.0
HIS	4.0	4.0	3.9	3.8
ILE	6.1	6.3	6.4	6.5
LEU	11.2	11.6	12.0	12.4
PHE	7.5	7.9	8.2	8.6
TYR	5.1	5.3	5.5	5.7
VAL	7.5	7.7	8.0	8.2
NE Pigs (MJ/kg)	8.91	8.91	8.90	8.90
SMET:SLYS	3.1	3.1	3.1	3.1
SM+C:SLYS	6.9	6.9	7.0	7.0
STHR:SLYS	6.6	6.6	6.5	6.5
STRP:SLYS	2.5	2.4	2.2	2.1
SHIS:SLYS	5.1	4.9	4.6	4.4
SILE:SLYS	7.7	7.6	7.5	7.5
SLEU:SLYS	13.9	14.0	14.1	14.3
SVAL:SLYS	9.2	9.1	8.9	8.8
SP+T:SLYS	16.0	16.4	16.8	17.2

Appendix 3: Analysed composition of diets

		GROWER, W-DDGS g/kg diet				FINISHER, W-DDGS g/kg diet			
		0	100	200	300	0	100	200	300
Code (FC0 11)		2331	2332	2333	2334	5553	5554	5555	5556
Ash	g/kg	50	42	48	50	54	50	49	55
Calcium	g/kg	8.1	5.3	8.2	8.9	9.7	7.8	7.8	9.1
Copper	mg/kg	18		21	25	24	24	17	23
Crude Fibre	g/kg	39	41	39	38	46	47	48	48
Crude Protein (N x 6.25) (Dumas)	g/kg	167	162	192	199	163	180	178	197
Magnesium	g/kg	1.5		1.6	1.5	1.5	1.6	1.5	1.6
Manganese	mg/kg	64		74	59	57	83	56	75
Moisture	g/kg	120	120	123	120	131	130	126	123
Neutral Detergent Fibre	g/kg	121	122	138	145	126	134	154	172
Oil A (Ether Extract)	g/kg	27.30	33.10	44.70	54.10	27.10	36.40	43.00	46.70
Phosphorus	g/kg	4.40	4.00	4.30	4.00	4.10	4.40	4.10	4.10
Potassium	g/kg	7.00	6.60	6.80	6.80	7.30	7.60	6.90	7.40
Salt (as NaCl)	g/kg	4.70	4.90	4.70	4.70	5.60	5.30	4.80	4.90
Sodium	g/kg	2.40	2.60	2.20	2.00	2.20	2.30	2.30	4.20
Starch	g/kg	329	318	331	340	383	366	363	318
Sugar as Sucrose	g/kg	38.4	32.0	33.1	32.0	36.3	35.7	33.5	27.6
Total Oil (Oil B)	g/kg	33.4	38.0	52.3	61.2	34.5	45.1	50.5	57.1
Zinc	mg/kg	99		106	92	91	121	92	105

Raw material basic data (provided by Premier)

Code : 309 Name : wDDGS ensus 2012 illinois AA

Analysis

[VOLUME]	%	100.0	PI NEGR MJ	MJ/kg	8.65	PI DEGR MJ	MJ/kg 12.18
DM	%	90.7	PI NESW MJ	MJ/kg	9.43	PI DESW MJ	MJ/kg 13.25
CR PROT	%	34.6	PI IDPRO	%	28.56		
OIL A	%	4.7	PI IDLYS	%	0.143462		
OIL B	%	7.5	SATS	%	0.87	PI IDMETH	% 0.318445
CR FIB	%	9.4	UNSATS	%	3.5	PI IDCYS	% 0.40608
NDF	%	26.5	UNSAT/SAT	%	4.022989	PI IDM+C	% 0.724282
ADF	%	7.9	CA	%	0.1	PI IDTHRE	% 0.587508
STARCH	%	2.5	PHOS	%	0.6	PI IDTRYP	% 0.203787
SUGAR	%	0.5	K	%	1.0	PI IDISOL	% 0.840348
ST + SU	%	3.0	NA	%	0.38	PI IDVAL	% 0.940317
GE KCAL/KG	Kcal/kg	4419.0	CL	%	0.35	PI IDLEU	% 1.622999
GE MJ/KG	MJ/Kg	18.49	SALT	%	0.57	PI IDP+T	% 1.63
ASH	%	4.5	MG	%	0.23	PI IDHIST	% 0.426106
LYS	%	0.52938	S	%	0.28	PI IDARG	% 0.925688
METH	%	0.46018	CU	mg/kg	12.0	PI DPHOS	% 0.42
CYS	%	0.59858	I	mg/kg	0.27	PI DPHOSH	% 0.42
M+C	%	1.05876	CO	mg/kg	0.06	VOLUME	% 100.0
THRE	%	0.97918	SE	mg/kg	0.1	PI D MET:L	%/% 2.219717
TRYPT	%	0.33908	ZN	mg/kg	90.0	PI D M+C:L	%/% 5.048598
ISOLEU	%	1.16	MN	mg/kg	63.0	PI D TRE:L	%/% 4.095217
VAL	%	1.1591	PI D TRY:L	%/%	1.420495		
LEU	%	1.46	PI D ISO:L	%/%	5.857635		
P+T	%	2.62268	PI D VAL:L	%/%	6.554467		
HIST	%	0.61934	PI D LEU:L	%/%	11.313093		
ARG	%	1.25944	PI D P+T:L	%/%	11.361894		
PI D HIS:L	%/%	2.970166	PI D MET:L	%/%	0.781818		
PI D ARG:L	%/%	6.452496					
PI CA:DP H	%/%	0.238095					
PI DL:DE G	%/MJ	0.011778					
PI DL:DE S	%/MJ	0.010827					

Appendix 4 Compositions of DDGS and of fractions produced by dry fractionation using combinations of sieving and elutriation. Codes are identified in Table 7.1.

Particle Class	Crude Protein (%)	Crude Fibre (%)	Neutral Detergent Fibre (%)	Oil B (%)	Ash (%)
DDGS	33.0%	8.08%	39.6%	7.26%	4.34%
L1	30.1%	9.69%	43.3%	6.13%	4.10%
M1	33.1%	8.60%	41.8%	7.34%	4.25%
H1	34.9%	6.74%	39.3%	7.35%	4.23%
L2	31.4%	9.35%	42.8%	6.76%	4.12%
M2	33.9%	7.66%	41.7%	7.52%	4.33%
H2	34.9%	6.39%	38.0%	7.25%	4.29%
F	31.9%	9.25%	37.4%	6.85%	4.42%
M	32.6%	8.55%	39.0%	7.18%	4.32%
C	33.9%	7.24%	37.6%	7.11%	4.42%
LL	30.8%	9.23%	40.2%	6.93%	4.62%
ML	32.2%	8.18%	37.5%	7.69%	4.89%
LM	32.1%	8.59%	39.7%	7.85%	4.69%
MM	33.3%	7.83%	37.9%	7.78%	4.72%
HM	34.7%	6.92%	36.7%	7.41%	4.81%
MH	34.5%	6.61%	37.4%	7.59%	4.81%
HH	34.5%	6.61%	37.1%	7.36%	4.51%
LF	31.4%	8.98%	38.1%	6.55%	4.59%
MF	32.3%	8.67%	38.3%	7.67%	4.39%
LM	29.7%	9.47%	32.4%	6.72%	4.39%
MM	32.9%	7.79%	37.9%	7.58%	4.20%
MC	32.9%	7.21%	35.2%	7.35%	4.51%
HC	34.1%	6.41%	33.3%	7.27%	4.51%
FL	31.2%	8.73%	37.4%	7.17%	4.27%
CL	28.0%	9.81%	41.8%	7.10%	4.06%
FM	32.5%	7.36%	32.7%	7.87%	4.38%
CM	33.3%	7.08%	35.5%	7.13%	4.29%
FH	34.3%	6.38%	35.3%	6.59%	4.49%
CH	33.9%	6.78%	36.3%	7.11%	4.29%

Appendix 5: Summary of Pig flow and Morbidity in HAU W-DDGS Pig Growth Trial

Treatment			**0**	**10**	**20**	**30**	**Total**
n pens			**9**	**9**	**9**	**9**	
Selection			207	207	207	207	828
Period 1	Grower	Removed	2	1	3	3	9
		Died	1	2	1	1	5
Period 2-4	A&B	Removed	10	9	12	5	36
		Died	6	3	3	2	14
Slaughter		Too small	1	2	4	0	7
Total Recorded at Slaughter			187	190	184	196	757
Batch			1	2	3		
Selection			276	276	276		
Period 1	Grower	Removed	2	5	2		
		Died	3	2	0		
Period 2-4	A&B	Removed	14	13	9		
		Died	3	6	4		
Slaughter		Too small	0	6	1		
Total Recorded at Slaughter			254	244	260		

Pre Treatment performance of HAU growth trial pigs 25-40 kg. (Pen data analysis)

Treatment		**0**	**10**	**20**	**30**	**sed**	**P value**
n pens		**9**	**9**	**9**	**9**		
Average Weight	**Age Weeks**						
St Wt	9	25.07	25.11	24.99	25.03	0.099	0.678
End Wt	12	39.64	39.68	39.6	39.67	0.358	0.996
	Diet						
Daily Feed Intake g/d	Grower	1316	1346	1340	1327	24.1	0.604
Daily Gain g/d	Grower	721	724	719	722	18.6	0.995
FCR	Grower	1.83	1.87	1.86	1.83	0.037	0.680

Appendix 6: Interactive effect of DDGS and Sex on growth and slaughter characteristics of pigs

This shows the initial slaughter data and is presented on the individual pig basis given that pen were mixed sex. The data as expected show significant differences between the sexes and the same growth trends as above. There was only one significant interaction between growth and sex. In general the slaughter data in relation to sex is as expected and there were no treatment effects shown in P2 and KO. Correlations between growth and FCR and P2 may be useful.

The effect of DDGS and Sex on growth and slaughter characteristics of pigs

		Average Weight				Daily Gain			
Treatment	**Sex**	**Period 1**	**Period 2**	**Period 3**	**Slaughter**	**Period 1**	**Period 2**	**Period 3**	**Slaughter**
0	**B**	39.45	56.56	81.41	99.8	732	805	876	986
10		39.53	58.83	84.53	103.2	736	906	907	998
20		39.50	57.94	82.56	100.5	740	867	869	960
30		39.96	59.34	86.31	105.2	747	910	951	1013
0	**G**	39.71	57.15	81.71	98.3	730	821	866	886
10		39.73	58.27	83.20	99.8	725	871	880	887
20		39.51	57.00	80.94	97.6	718	820	845	891
30		39.62	57.57	82.87	99.3	730	843	893	879
P Value		0.922	0.332	0.185	0.157	0.896	0.025*	0.324	0.306
SEM		0.693	0.946	1.244	1.443	20.8	20.2	19.6	25.5

Interactive effect of DDGS and sex on the slaughter and post slaughter characteristics of pigs

DDGS Inclusion	Sex	Liveweight (kg)	Hot weight (kg)	P2 (mm)	Kill out %	Lean meat %	Cold Weight	Drip Loss%	pH45	pH24
0	B	100.8	76.73	10.15	76.13	62.08	75.19	0.25	6.582	5.534
	G	99.0	77.80	10.29	78.56	62.01	76.25	0.25	6.570	5.512
10	B	103.3	79.42	10.57	76.90	61.86	77.83	0.14	6.619	5.534
	G	100.4	79.29	10.79	79.00	61.64	77.70	0.69	6.624	5.485
20	B	101.8	77.63	10.80	76.31	61.52	76.08	0.29	6.588	5.570
	G	98.1	76.63	10.34	78.13	61.89	75.09	0.24	6.593	5.490
30	B	105.1	80.51	10.69	76.59	61.82	78.90	0.28	6.577	5.500
	G	99.4	78.05	10.46	78.54	61.87	76.49	0.26	6.575	5.517
Treatment*Sex		0.218^{NS}	0.131^{NS}	0.266^{NS}	0.527^{NS}		0.131^{NS}	0.283^{NS}	0.973^{NS}	0.351^{NS}
Treatment		0.022^{*}	0.002^{**}	0.100^{NS}	0.004^{**}		0.002^{**}	0.745^{NS}	0.057^{NS}	0.903^{NS}
Sex		$<0.001^{***}$	0.227^{NS}	0.548^{NS}	$<0.001^{***}$		0.227^{NS}	0.319^{NS}	0.924^{NS}	0.112^{NS}
CV%		9.14	9.39	17.48	2.56		9.39	559.79	2.64	4.18

NB. As with Table 6 in the main report, only clean slaughtered pigs were included in this analysis of slaughter and post slaughter characteristics.

Interactive effect of DDGS and sex on the slaughter and post slaughter characteristics of pigs

DDGS Inclusion	Sex	Liveweight (kg)	Hot weight (kg)	P2 (mm)	Kill out %	Lean meat %	Cold Weight	Drip Loss%	pH45	pH24
0	B	100.8	76.73	10.15	76.13	62.08	75.19	0.25	6.582	5.534
	G	99.0	77.80	10.29	78.56	62.01	76.25	0.25	6.570	5.512
10	B	103.3	79.42	10.57	76.90	61.86	77.83	0.14	6.619	5.534
	G	100.4	79.29	10.79	79.00	61.64	77.70	0.69	6.624	5.485
20	B	101.8	77.63	10.80	76.31	61.52	76.08	0.29	6.588	5.570
	G	98.1	76.63	10.34	78.13	61.89	75.09	0.24	6.593	5.490
30	B	105.1	80.51	10.69	76.59	61.82	78.90	0.28	6.577	5.500
	G	99.4	78.05	10.46	78.54	61.87	76.49	0.26	6.575	5.517
Treatment*Sex		0.218^{NS}	0.131^{NS}	0.266^{NS}	0.527^{NS}		0.131^{NS}	0.283^{NS}	0.973^{NS}	0.351^{NS}
Treatment		0.022^{*}	0.002^{**}	0.100^{NS}	0.004^{**}		0.002^{**}	0.745^{NS}	0.057^{NS}	0.903^{NS}
Sex		$<0.001^{***}$	0.227^{NS}	0.548^{NS}	$<0.001^{***}$		0.227^{NS}	0.319^{NS}	0.924^{NS}	0.112^{NS}
CV%		9.14	9.39	17.48	2.56		9.39	559.79	2.64	4.18

NB. As with Table 6 in the main report, only clean slaughtered pigs were included in this analysis of slaughter and post slaughter characteristics.