

## Grange Beef Research Centre

### ENVIRONMENT AND ALTERNATIVE FORAGE USE

#### **The impact of incubation duration on methanogenesis of four diets assessed using the Rumen Simulation Technique (RUSITEC)**

This experiment was designed to assess the impact of reducing the incubation period (14 vs. 21 d) for a range of diets when using the RUSITEC technique. The RUSITEC technique was operated as described by Czerkawski and Breckenridge (1977). The methanogenic potential of four contrasting diets [grass hay + wheat grain at 50:50 on a dry matter (DM) basis, perennial ryegrass, red clover and grass silage] was assessed during two incubation periods (14 vs. 21 d). Two sets of RUSITEC apparatus (Sanshin Industrial Co. Ltd., Japan), each with 8 incubation vessels (each of 850 ml capacity), were used. Four replicates per diet, two per set, were assigned randomly. Each vessel was fed daily with a nylon bag containing 10.2 g DM of the appropriate diet, and these bags were replaced after 48 h incubation. Vessels were immersed in a water bath at 39°C. On day 1, rumen liquor and solid digesta were collected from two rumen fistulated steers fed on grass silage, pooled and placed in the vessels as an initial inoculum. Artificial saliva was supplied to each vessel at a flow rate of 25 ml/h using a peristaltic pump. Total daily gas production was measured, and gas and overflow liquid samples were taken, during days 10 to 14 and 17 to 21, for 14 d and 21 d incubation periods, respectively. Total volatile fatty acid (tVFA) and CH<sub>4</sub> concentrations were determined by gas chromatography (Shimadzu GC-17A with FID detector) and ammonia (NH<sub>3</sub>) was measured using the Thermo Electron Kinetic method for plasma ammonia (Olympus AU400 Analyzer). Apparent dry matter digestibility (DMd) was measured as disappearance of DM during a 48 h incubation. Results from each vessel were averaged for days 10–14 and for days 17–21. Data were subjected to a repeated measures procedure, with diet, duration of incubation, apparatus, and diet x duration of incubation interaction included in the model as fixed factors. Data from one vessel were omitted due to a system malfunction in that vessel. All statistical analyses were conducted using the PROC MIXED procedure in SAS version 9.1. Multiple comparisons among means were performed using the Tukey-Kramer's procedure.

The main results are summarised in Table 50. Methane output was lower while total gas production was higher ( $P < 0.05$ ) after 14 d compared to 21 d of incubation, but no interaction ( $P > 0.05$ ) with diet was detected. Dry matter disappearance was affected ( $P < 0.05$ ) by incubation duration, with the value for ryegrass being significantly higher after 14 than 21 days duration; an interaction between diet and duration of incubation was detected ( $P < 0.05$ ). A decrease in tVFA production ( $P < 0.001$ ) occurred with the longer duration of incubation and this partially explains the corresponding decline in total gas production. Ammonia production as a result of protein degradation by rumen microbes did not differ ( $P > 0.05$ ) between the two durations of incubation. Diet significantly affected all variables measured. The high starch content diet, hay + wheat, and the red clover produced more CH<sub>4</sub>/g digested ( $P < 0.05$ ) than either ryegrass or grass silage. Even if the finding of the high starch diet producing more CH<sub>4</sub> than grass or grass silage was unexpected, it is not unprecedented and agrees with previous findings (Klevenhusen *et al.*, 2008).

It is concluded that although CH<sub>4</sub> yield differed significantly with duration of incubation the absence of an interaction between diet and duration of incubation gives encouragement that studies on methanogenesis with contrasting diets can be undertaken with a 14 d rather than a 21 d incubation period. The high starch diet (hay + wheat) and the red clover had a higher methanogenic potential than either grass or grass silage when assessed using the RUSITEC.

**Table 50: Rusitec fermentation characteristics for four diets after 14 and 21 days incubations**

Variable	Diets <sup>1</sup>				SEM <sup>2</sup>			P-value <sup>2</sup>		
	H+W	L	G	S	Dur.	Diet	DxD	Dur.	Diet	DxD
CH <sub>4</sub> ml/g dig					2.9	4.1	5.8	0.0022	<0.001	0.312
14 d	54	48	42	38						
21 d	55	52	42	42						
TGP ml/g dig					0.9	1.3	1.8	0.029	<0.001	0.133
14 d	247	218	203	206						
21 d	237	222	193	199						
DMD g/g					0.0029	0.0042	0.0059	0.029	<0.001	0.017
14 d	0.660	0.721	0.785	0.765						
21 d	0.670	0.705	0.817	0.782						
tVFA mmol/d					2.1	2.9	4.1	<0.001	0.035	0.123
14 d	134	123	139	151						
21 d	118	114	118	127						
NH <sub>3</sub> mg /g					0.9	1.3	1.8	0.427	<0.001	0.241
14 d	9	17	30	10						
21 d	8	14	31	9						

TGP = total gas production; CH<sub>4</sub> = methane production; DMD = dry matter disappearance; tVFA total volatile fatty acids; NH<sub>3</sub> = ammonia.

<sup>1</sup>H+W = Hay + wheat 50:50 on dry matter basis, L = red clover, G = ryegrass and S = grass silage.

<sup>2</sup>Dur. = Duration of incubation 14 vs.21 d, DxD = duration of incubation x diet type interaction.

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### **Methane emissions of finishing beef cattle offered whole-crop wheat silage differing in grain to straw ratio**

One strategy for reducing emissions of enteric methane (CH<sub>4</sub>) from cattle is to increase the starch and reduce the fibre content of the diet. The associated expected improvement in the rate of growth should further reduce CH<sub>4</sub> emissions per unit of weight gain. This study examined the effects of altering the ratio of grain to straw in whole-crop wheat silage (WCW) on CH<sub>4</sub> emissions and associated performance parameters of finishing beef steers, and ranked these relative to grass silage and *ad libitum* concentrates.

Ninety continental cross-bred steers of mean initial live weight 538 (s.d. 27.6) kg were blocked on weight and assigned to one of six dietary treatments in a randomised complete block design. The WCW was ensiled, subsequently separated into grain and straw and these were, in turn, re-ensiled separately. Four grain to straw ratios were formulated at feedout: (I) 0:100, (II) 12:88, (III) 26:74 and (IV) 45:55. All four WCW treatments as well as a grass silage (GS) based diet (negative control) were each offered *ad libitum* and supplemented with 3 kg concentrates daily per animal. A sixth treatment, *ad libitum* concentrates (ALC) supplemented with 5 kg grass silage daily per head, was used as a positive control. Daily CH<sub>4</sub> emissions were measured using the SF<sub>6</sub> tracer technique. Diets were offered individually for 154 days after which animals were slaughtered and carcass data recorded. Data were analysed using the GLM procedure in Statistical Analysis Systems) and the model included terms for treatment and block. The response to

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incremental increase in grain to straw ratio for WCW was tested using orthogonal polynomial contrasts.

The mean chemical composition and particle length distribution of WCW I to IV, and of GS, are summarised in Table 51. Increasing the ratio of grain to straw in WCW had a quadratic effect on total daily and DM intake corrected enteric CH<sub>4</sub> emissions, but linearly reduced g CH<sub>4</sub>/kg carcass gain (Table 52). Animals on GS had the highest g CH<sub>4</sub>/kg DM intake and ALC had the lowest ( $P<0.05$ ) output of methane in all three units of measurement. A quadratic response in forage and total DM intake was observed as grain to straw increased in WCW, while intake for GS was lowest ( $P<0.001$ ) overall. Liveweight gain was higher ( $P<0.05$ ) for ALC than any of the forage treatments. Carcass weight and carcass gain increased with increasing grain in the WCW treatments and were similar to GS, with all forage treatments having lower values for both variables compared with ALC.

It is concluded that increasing the ratio of grain to straw in whole-crop wheat silage had a quadratic effect on CH<sub>4</sub> emissions when expressed either as g/day or g/kg DM intake, but resulted in a linear decrease when expressed as g/kg carcass gain. The ALC diet consistently reduced CH<sub>4</sub> emissions compared to forage based diets while the effect of grass silage varied with the units of measurement in which CH<sub>4</sub> emission was expressed. Liveweight and carcass gains increased with increasing grain to straw in WCW and were lower than ALC but similar to grass silage.

**Table 51: Chemical composition and particle length of WCW and grass silages (mean (SD))**

	WCW treatment				GS
	I	II	III	IV	
Chemical composition					
DM <sup>1</sup> (g/kg)	448 (14.4)	489 (16.8)	536 (14.4)	574 (12.8)	238 (18.2)
DMD <sup>2</sup> (g/kg)	612 (22.0)	659 (16.9)	706 (17.6)	761 (26.9)	793 (28.5)
Starch (g/kg DM)	155 (22.2)	268 (26.1)	353 (29.1)	436 (31.7)	-
NDF <sup>3</sup> (g/kg DM)	524 (25.5)	444 (15.2)	379 (19.5)	310 (28.8)	513 (17.4)
Particle length (g DM/kg DM)					
0 – 25 mm	789 (49.3)	853 (35.7)	884 (1.2)	897 (32.8)	176 (10.2)
26 – 50 mm	111 (19.5)	99 (13.0)	81 (2.9)	55 (18.0)	258 (9.1)
51 – 75 mm	74 (62.1)	28 (12.7)	21 (2.7)	29 (2.4)	207 (2.1)
76 – 100 mm	15 (10.3)	16 (11.9)	7 (2.3)	8 (2.5)	169 (5.5)
> 100 mm	11 (7.01)	4 (1.5)	7 (2.5)	12 (8.0)	189 (2.3)

<sup>1</sup>Uncorrected for volatiles; <sup>2</sup>Dry matter digestibility, measured in vitro; <sup>3</sup>Neutral detergent fibre.

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**Table 52: Methane emissions, intake and performance of finishing steers**

	WCW		Treat <sup>1</sup>		GS	ALC <sup>2</sup>	s.e.m. <sup>3</sup>	Significance		
	I	II	III	IV				Treat	Lin <sup>†</sup>	Quad <sup>†</sup>
Forage intake (kg DM/day)	7.7	8.7	8.6	8.3	6.1	1.2	0.20	***	NS	**
Total intake (kg DM/day)	10.3	11.2	11.2	10.8	8.7	10.8	0.22	***	NS	**
Live weight gain (g/day)	820	1046	1103	1043	929	1335	62.5	***	*	*
Carcass gain (g/day)	577	650	765	757	664	915	33.9	***	***	NS
Carcass weight (kg)	359	370	387	386	372	410	5.3	***	***	NS
FCR <sup>4</sup>	18.3	18.4	14.8	14.7	13.3	12	0.76	***	***	NS
CH <sub>4</sub> g day	260	315	321	249	312	168	21.5	***	NS	***
CH <sub>4</sub> g/ kg DMI	26.5	27.8	28	23.1	35.5	14.4	1.95	***	*	**
CH <sub>4</sub> g/ kg carcass gain	483	472	408	323	486	185	36.5	***	**	NS

<sup>1</sup>Treatment; <sup>2</sup>Includes intakes during adaptation; <sup>3</sup>s.e.m. for n = 15; <sup>4</sup>Feed conversion ratio expressed as kg DM intake/kg carcass gain; NS = Non-significant. <sup>†</sup> Linear, Quadratic, WCW only. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

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### **GreenGrass: Developing grass for sustainable, renewable energy generation and value-added products**

Field plots (each 10m x 2m) were marked out in a fine soil tilth. There were 70 plots per replicate block and three blocks. A split-split plot design was used, with the main plots being harvest date in the primary growth (12 May, 26 May, 9 June, 23 June and 7 July), sub plot being the rate of inorganic N fertiliser applied in March (0 vs. 125 kg N/ha) and sub-sub plot being the herbage species sown: Italian Ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), red clover (*Trifolium pratense*) and an old permanent pasture mixture. These plots were conditioned during 2008 and will be used in experiments starting in May 2009.

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### **The quality of forage maize pre- and post-ensilage**

Field plots were sown (split-plot design) in May (each plot was 25m long; 3 replicate blocks) with the following maize varieties (sub-plots): Tassilo (conventional), Beethoven (conventional), Nescio (cold tolerant), Andante (cold tolerant), Atletico (KXA 4171; high biomass) and KXA 7211 (high biomass). Plots were harvested (main plots) in mid September, early October and late October. Yields of whole crop, cob and stover were obtained, and each was sampled separately,

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and ensiled separately in laboratory silos. A range of physical, chemical and microbiological measurements will be undertaken.

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