

**SHEEP PRODUCTION**

**Effects of extended grazing, grass silage feed value and winter shearing on ewe and lamb performance**

Previous studies at Athenry have clearly shown that extended (winter) grazing summer-shorn ewes in mid, late or throughout pregnancy increased lamb birth weight and weaning weight (Research Report 2005, pp 112-113). Furthermore, previous studies at Athenry showed that shearing March-lambing ewes in mid December induced an increase in lamb birth weight similar to that achieved from extended grazing ewes for the duration of pregnancy (Research Report 2005, pp 112-113). The aims of this study were to evaluate the effects of extended grazing, herbage allowance in mid pregnancy; silage feed value and winter shearing of ewes on ewe performance in mid and late pregnancy, and on lamb birth weight and subsequent growth rate.

A total of 120 crossbred ewes (60 first and 60 second crop; initial weight 67.6 kg, condition score 3.7) were allocated to the 6 treatments (2 silage feed values x 2 shearing treatments plus 2 grass allowances in mid pregnancy) in a randomised design study from 17 December to lambing in mid March. Medium (precision chopped) and low (big baled) feed value grass silages were ensiled on 30 May and 5 August, respectively. Predominantly perennial ryegrass permanent swards, which had been harvested for silage on 6 September received fertiliser N (34 kg/ha) for extended grazing. The pasture was grazed *in situ* at DM allowances of 1 or 1.8 kg/head daily from 1 December to 1 February. From 1 February the ewes were allocated DM allowances of 1.5, 1.6, 2.0, 2.0 and 2.0 kg/head per day during weeks 6, 5, 4, 3 and 2 prior to lambing, respectively. During the week prior to parturition the ewes were “spread out” for lambing. Indoors the grass silages were offered as the sole forage *ad-libitum* up to lambing. Half of the housed ewes were shorn at the beginning of the study. All ewes were offered daily 0.3, 0.5, 0.6, 0.7, 0.8 and 0.8 kg concentrate/head during weeks 6, 5, 4, 3, 2 and 1 prior to lambing. The data were analysed using PROC GLM and PROC MIXED procedures of SAS. *A priori* contrasts were used to evaluate the effects of herbage allowance, shearing and forage type. Ewes rearing triplets received a daily allowance of 1 kg concentrate until April 30 and their lambs were offered concentrate supplementation to a maximum of 300 g/head daily until weaning at 14 weeks.

**Table 1: Effect of extended grazing, shearing and silage feed value on animal performance**

Variable	Treatment (T)								Significance		
	EG <sup>1</sup> (kg/DM/day)			Housed				s.e.	S v US	S v EG	US v EG
	Low	High	s.e.	Shorn (S)		Unshorn (US)					
			<sup>1</sup> LFVS	MFVS	LFVS	MFVS					
Ewe CS at lambing	2.95	3.10	0.172	3.02	3.19	3.29	3.32	0.126	NS	NS	*
Litter size	1.70	1.62	0.204	1.77	1.87	1.75	1.93	0.125	NS	NS	NS
Number reared	1.53	1.42	0.119	1.65	1.65	1.74	1.67	0.146	NS	NS	NS
Lamb performance:											
Birth weight (kg)	4.31	4.48	0.12	4.54	4.46	4.08	3.79	0.146	***	NS	***
Growth rate (g/d)	295	298	7.0	298	299	281	287	8.2	P=0.06	NS	P=0.09
Weaning wt (kg)	33.2	33.7	0.73	33.8	33.6	31.6	31.8	0.9	*	NS	*

<sup>1</sup> LFVS = low feed value silage, MFVS = medium feed value silage, EG = extended herbage allowance in mid pregnancy.

<sup>2</sup> There were no (P>0.05) extended grazing herbage allowance, silage feed value effects or silage X shearing interactions.

The swards had mean herbage DM mass of 3373 kg/ha. The low and medium feed value grass silages and extended grazed herbage had DM and predicted metabolisable energy concentrations of 225 and 265 and 132 g/kg; and 10.3, 10.9 and 10.4 MJ/kg DM, respectively.

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Grass silage feed value did not alter silage intake. Shearing indoors increased ( $P < 0.05$ ) silage dry matter intake (0.68 and 0.80 kg/day for the unshorn and shorn ewes, respectively).

The effects of extended grazing, shearing and silage feed value on animal performance are presented in Table 1. Relative to the housed unshorn ewes, shearing and extended grazing increased lamb birth weight ( $P < 0.001$ ) and weaning weight ( $P < 0.05$ ), and tended to increase lamb growth rate from birth to weaning. Unshorn ewes indoors had a higher ( $P < 0.05$ ) body condition score at lambing than extended-grazed ewes. Herbage allowance in mid pregnancy or silage feed value did not alter ewe or subsequent lamb performance. Treatment had no effect on lambing assistance score. As determined by lamb weaning weight, a DM allowance of 0.8 kg per day of either the low- or medium-feed-value grass-silage had the same feed value as 1.8 kg DM of extended grazed herbage allowance offered in mid and late pregnancy. There were no interactions between silage feed value and shearing treatment.

An analysis of the relationship between lamb birth weight and weaning weight yielded a significant linear relationship ( $P < 0.01$ ) as described by the following equation;

$$WW = 19.45 + 3.16 BW \text{ (s.e. } 0.6559^{**}) \quad R^2 = 0.85$$

where WW = weaning weight (kg) and BW = birth weight (kg)

It is concluded that shearing housed ewes resulted in the same lamb birth weight and subsequent performance as outwintering unshorn ewes and lambing outdoors. The increased lamb performance from ewes lambing outdoors, relative to indoors unshorn, in this and previous studies is probably due to elimination of heat stress rather than increased nutrient intake from extended grazing.

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*RMIS No. 4925*

### **The effects of herbage allowance and frequency of allocation, and silage feed value on lamb birth weight and subsequent performance when offered to ewes in mid gestation**

It is essential to improve efficiency and reduce costs of production with decoupling of subsidy from production post Mid Term Review of the Common Agricultural Policy. Recent studies at Athenry have shown that extended grazing (grazing during winter), either during mid, late or throughout pregnancy, provides a low cost system of wintering ewes (Research Report 2005, pp 112-113). The aims of this study were to evaluate the effects of herbage allowance and frequency of allocation, and potential interactions during mid gestation on ewe performance and lamb birth weight and subsequent performance. Furthermore a direct comparison of herbage allowance and grass silages of differing feed value was also included.

Swards which were harvested for silage on 6 September received fertiliser N (34 kg/ha) for extended grazing. The pasture was grazed *in situ* at dry matter (DM) allowances of 1.0 and 1.8 kg/head per day. The herbage was allocated daily or twice weekly. Medium (precision chopped) and low (big baled) feed value grass silages were ensiled on 30 May and 5 August, from the primary growth and regrowth, respectively. The silages were offered *ad libitum* as the sole forage. The study involved 120 ewes (initial live weight 74.1 kg; condition score 3.7) from 7 December to 1 February in a fully randomised design and was analysed using Proc GLM and Proc MIXED procedures of SAS. On 1 February all ewes were housed and offered the medium feed value grass silage supplemented with concentrate at a total of 19 kg per head up to lambing. The ewes were synchronised to yield lambs on 27 February. All ewes rearing single or twin lambs were grazed together until weaning without concentrate supplementation. Ewes rearing triplets were offered 0.5 kg concentrate daily whilst the lambs received concentrate supplementation to a maximum of 300 g day. The lambs were weaned at 14 weeks.

The low and medium feed value grass silages and extended grazed grass had DM and predicted metabolisable energy concentrations of 225, 265 and 132 g/kg, 10.3, 10.9 and 10.4 MJ/kg, respectively. The swards had a mean herbage DM mass of 3373 kg/ha. Grass silage feed value did not alter forage dry matter intake (0.93 and 0.91 kg/day for the low and medium feed value grass silages, respectively). Increasing herbage allowance increased ( $P<0.05$ ) organic matter intake (0.42 and 0.58 kg/day for the low and high allowance, respectively) but decreased herbage organic matter utilisation (0.46 and 0.35 for the low and high allowance respectively). Frequency of herbage allocation did not alter ( $P>0.05$ ) organic matter intake or utilisation. The effects of herbage allowance and frequency of allocation and silage feed value on animal performance are presented in Table 2. Increasing herbage allowance significantly ( $P<0.001$ ) increased ewe live weight and condition score at the end of extended grazing and tended ( $P=0.06$ ) to increase condition score at lambing. Weight of lambs at birth and weaning and daily live-weight gain were significantly increased due to increased herbage allocation. Herbage allowance did not alter ( $P>0.05$ ) ewe condition score or weight at weaning or litter size. Frequency of herbage allocation or grass silage feed value did not alter ewe or lamb performance. However, there was a significant interaction between herbage allowance and frequency of allocation for ewe condition score and weight at weaning, lambing assistance, and lamb birth weight. At the low allowance reducing frequency of allocation reduced ewe weaning condition score and weight while at the high allowances reducing frequency of allocation increased ewe weaning condition score and weight. Reduced frequency of allocation increased lambing assistance and lamb birth weight at the low herbage allowance but reduced lambing assistance and lamb birth weight at the high allowance. As determined by lamb weaning weight, 0.92 kg dry matter of the low and medium feed value grass silages had the same feed value as 1.4 and 1.5 kg DM of extended grazed herbage allocated daily, respectively.

**Table 2: Effect of herbage allowance and frequency of allocation, and silage feed value in mid pregnancy on animal performance**

	Forage						s.e.	Significance <sup>§</sup>			
	Grass (G) allowance (A) (kg DM/d)				Grass silage feed value(S)			GA	F	GA x F	S v G
	1.0	1.0	1.8	1.8	Low	High					
Frequency of movement	Daily	Twice weekly	Daily	Twice weekly	Low	High					
Ewe condition score at:											
end of grazing	3.08	2.90	3.38	3.32	3.19	3.25	0.086	***	NS	NS	
lambing	2.91	2.75	3.09	3.06	3.14	3.07	0.127	P=0.06	NS	NS	
weaning	3.33	2.98	2.97	3.10	3.12	3.10	0.109	NS	NS	*	NS
Ewe weight (kg) at:											
end of grazing	69.5	67.2	74.4	73.6	73.2	74.7	0.81	***	P=0.06	NS	
weaning	76.8	72.3	73.5	75.1	77.2	75.2	1.43	NS	NS	*	NS
Litter size	1.72	2.10	1.99	1.71	1.84	2.15	0.152	NS	NS	NS	
Lambing assistance (%)	11	40	19	6	11	12		NS	NS	*	NS
Lamb performance											
Weight (kg) at:											
birth	4.26	4.68	5.12	4.73	4.52	4.50	0.138	**	NS	**	
weaning	32.8	34.4	36.0	35.1	34.2	34.7	0.87	*	NS	NS	NS
LWG (g/d)	287	301	313	308	312	315	7.7	*	NS	NS	

<sup>§</sup>Silage feed value (S) was not significant for any variable.

It is concluded that increasing herbage allowance during mid pregnancy increased lamb weaning weight and daily live-weight gain. The herbage allowance by frequency of allocation interaction for lamb birth weight may be explained by the effects of the combination of low feed allowance and twice weekly herbage allocations on maternal partitioning of nutrients to

foetal growth. Frequency of herbage allocation had no effect on herbage intake or animal performance. Offering 0.92 kg DM of the low and medium feed value grass silage had the same feed value as 1.4 and 1.5 kg DM of extended grazed herbage allocated daily, respectively.

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**The effects of grazing date and management of autumn pasture on subsequent herbage yield**

Recent studies at Athenry have shown that extended (winter) grazing ewes either in late or throughout pregnancy yields lambs with similar birth and weaning weights as from ewes shorn indoors (Research Report 2005, pp 112-113). Frequency of grass allocation (daily or twice weekly) in mid pregnancy had no effect on lamb birth weight or subsequent performance. Consequently, there are viable alternative strategies to housing, on low to moderately stocked farms, for maintaining the ewe in mid and late pregnancy by extended grazing. With the decoupling of subsidy from production post Mid Term Review of the Common Agricultural Policy it is essential to improve efficiency and reduce costs of production. The aim of the present study was to evaluate the effects of grazing (closing) date and management of autumn pasture on subsequent herbage regrowth.

Predominantly perennial ryegrass permanent swards, which were harvested for silage on 7 September received fertiliser N (34 kg/ha). The pasture was grazed *in situ* at daily dry matter (DM) allowances of 1.0 or 1.8 kg/head from 6 to 12 December, 27 December to 3 January and 17 to 23 January. Within each herbage allowance the herbage was allocated daily or twice weekly. Thus there were 12 “grazing treatment” blocks. Within each block representative plots were allocated at random for harvesting on 27 April (3 plots) or 25 May (3 plots). At grazing the swards had mean herbage DM mass and DM concentrations of 3373 kg/ha and 132 g/kg, respectively. The plots received 60, 24.5 and 105 kg/ha of N, P and K fertiliser, respectively, on 14 February. The plots were mowed with an Agria mower to a height of 2 cm, herbage was weighed and pre harvest grass heights were also determined. The data were analysed with blocks considered random and with fixed effects for herbage allowance, frequency of allocation, grazing date, harvest date and 2-factor interactions using Proc MIXED of SAS.

**Table 3: Effects of grazing date and management of autumn saved pasture, and harvest date on subsequent yield**

	HA <sup>1</sup>		FA		Grazing date (GD)			Harvest date		Significance <sup>2</sup>			
	(kg/day)		Daily	Twice weekly	12 Dec	3 Jan	23 Jan	(HD)		HA	FA	GD	HD
	1.0	1.8						27 Apr	25 May				
DM yield (t/ha)	2.79	3.93	3.24	3.43	4.49	3.43	2.27	1.64	5.62	**	NS	**	***
CI for DM yield	(2.37-3.25)	(3.42-4.46)	(2.78-3.73)	(2.97-3.94)	(3.84-5.20)	(2.86-4.05)	(1.81-2.78)	(1.47-1.83)	(5.29-5.95)				
Sward height (cm)	13.9	15.1	14.0	15.0	16.3	14.4	12.8	9.5	19.5	NS	NS	*	***

<sup>1</sup>HA = Herbage DM allowance, FA = Frequency of herbage allocation.

<sup>2</sup>There were no HA x GD, HA x FA interactions.

The effects of herbage allowance, frequency of allocation and grazing date of autumn pasture and subsequent harvest date on herbage height and DM yield are presented in Table 3. Dry matter yield was increased by increasing herbage grazing allowance (P<0.01), earlier grazing date (P<0.01) and delaying harvest date (P<0.001). Pre harvest sward height was increased due to earlier grazing date (P<0.05) and delayed harvest date (P<0.001). There were

significant herbage grazing allowance by harvest date ( $P < 0.01$ ) and frequency of herbage allocation by harvest date ( $P < 0.05$ ) interactions for sward height. The sward heights for the low and high allowances harvested on 27 April and 25 May were 8.01 and 10.94 cm, and 19.75 and 19.29 cm, respectively. The relationship between sward height and herbage yield differed ( $P < 0.001$ ) between harvest dates. For the 27 April and 25 May harvest dates each 1 cm change in sward height was equivalent to 284 and 83 kg DM in herbage yield, respectively. There were no other significant two way interactions.

There was a negative relationship between grazing date and subsequent herbage yield which is best described by the following relationship:

$$\text{HDMY} = -54.20 (2.207) X + 4573 (60.4) \quad (R^2 = 0.998)$$

where HDMY = herbage dry matter yield (kg/ha), X = days delay in grazing.

It is concluded each day delay in grazing autumn pasture and subsequent harvest date reduced and increased herbage DM yield by 54.2 and 147.2 kg respectively. There was a positive relationship between herbage height and yield at the early harvest date but not at the later harvest date.

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### **An evaluation of grass allocation management to single and twin bearing ewes in late pregnancy on ewe and subsequent lamb performance**

It is essential to improve efficiency and reduce costs of production following the recent decoupling of subsidy from production post Mid Term Review of the Common Agricultural Policy. The national average stocking and weaning rates are 8.7 ewes/ha and 1.33 lambs weaned per ewe put to the ram, respectively. Consequently, there are opportunities to reduce the costs of maintaining the ewe by extended grazing in late pregnancy. Previous studies at Athenry have clearly shown that extended grazing in late and throughout pregnancy produced lambs with similar birth weight and subsequent performance as ewes housed indoors, shorn and offered grass silage as the sole forage supplemented with similar levels of concentrate (Research Report 2005, pp112-113). The aim of the current study was to evaluate grass allocation management to single and twin bearing ewes during the last 8 weeks prior to lambing.

This study was undertaken on a commercial farm in Co. Kilkenny. A total of 152 ewes (initial live weight 65.2 (s.d. 10.1) kg and body condition score of 3.0 (s.d. 0.45) kg) were allocated to four treatments (2 litter sizes x 2 herbage allocation systems). Predominantly perennial ryegrass swards were closed in rotation and received fertiliser N (25 kg/ha) between mid September and early November for extended grazing between 30 January and 24 March. The ewes were scanned for litter size prior to the study. The pasture was grazed *in situ* by single- and twin-bearing ewes which were either grouped separately or grazed in a leader-follower system with twin bearing ewes leading and single-bearing ewes following. In the leader follower system the twin-bearing ewes received the combined herbage allocation which the twin and single bearing ewes grouped separately received, and the single bearing ewes grazed the residue. The daily herbage dry matter (DM) allowances for weeks 7 and 6, 5 and 4, 3 and 2, and prior to "spread out" were as follows: 1.3, 1.4, 1.6 and 1.6 kg for single bearing ewes grazed separately; 1.4, 1.6, 1.9 and 2.7 kg for twin bearing ewes grazed separately; 2.7, 3.0, 3.5 and 4.3 kg for the twin bearing ewes followed by the single bearing ewes in the leader-follower system. The ewes were spread out on 25 March for lambing between 25 March and 22 April. Ewes received no concentrate supplementation during the study. Pre and post grazing grass clips were taken to ground level once weekly to determine herbage allowance and intake. The data were analysed using general linear model procedures of SAS. *A priori* contrasts were used to test the effects of system of grass allocation and litter size. All ewes and lambs were grazed in one flock until weaning (19 July).

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The swards which were grazed pre-lambing had mean herbage height, DM mass and DM concentrations of 9.1 cm, 2349 kg/ha and 203 g/kg, respectively. From lambing to weaning the mean pre and post-grazing heights were 10.2 cm and 5.1 cm, respectively. The effects of grass allocation, management pre lambing and litter size on ewe and lamb performance are presented in Table 4. Single bearing ewes grazed in the leader follower system had lower ( $P<0.01$ ) condition score at lambing relative to those grazed separately. Grazing twin bearing ewes in a leader follower system increased herbage intake ( $P<0.001$ ) relative to those grazing separately. Twin lambs were lighter than singles ( $P<0.001$ ) at birth. Relative to ewes rearing singles those rearing twins had a lower ( $P<0.01$ ) condition score at weaning. System of grass allocation in late pregnancy did not alter ewe condition score at weaning, or lamb birth weight, growth rate or weaning weight.

**Table 4: Effect of grass allocation management and litter size on animal performance**

Litter size	Treatment				s.e.	Significance		
	Single		Twin			Single SvF	Twin SvL	Single v Twin
	Separate (S)	Follower (F)	Separate (S)	Leader (L)				
Grassland system								
Grass DMI (kg/d)	1.16	0.88	1.35	2.20		NS	**	**
Ewe condition score at:								
lambing	2.93	2.60	3.00	3.12	0.077	**	NS	NS
weaning	3.17	3.15	2.85	2.88	0.085	NS	NS	NS
Lamb performance								
Birth weight (kg)	5.97	5.80	4.80	4.95	0.142	NS	NS	NS
Growth rate (g/d)	266	269	224	228	7.5	NS	NS	***
Weaning wt (kg)	32.4	32.4	27.1	27.6	0.80	NS	NS	***

It is concluded that in a flock of single- and twin-bearing ewes, allocating herbage either daily or grazing in a leader-follower system (singles following twins) in late pregnancy did not alter lamb birth weight or subsequent growth rate. However single bearing ewes in the leader follower system had a lower condition score at lambing relative to ewes grazing separately.

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### The effects of herbage allocation and concentrate supplementation on the performance of replacement ewe lambs offered extended grazed pastures

One of the major costs in sheep production is the cost of rearing replacements. Extended grazing may reduce feed costs. Furthermore increasing the number of litters reared during the life time reduces replacement rate and subsequently costs. The aim of the present study was to evaluate the effect of herbage allowance and concentrate supplementation on animal performance during extended grazing and on compensatory growth during the subsequent grazing season. Furthermore the potential herbage allowance sparing effect of concentrate supplementation was also determined.

Old permanent, predominantly perennial ryegrass, pasture (12 ha) were topped to a sward height of 4.5 cm and received fertiliser N (38 kg/ha) on 8 September for grazing between 16 December and 3 March. The pasture was grazed *in situ* at herbage dry matter (DM) allowances of 0.75 (L), 1.25 (M) and 1.75 (H) kg/day as the sole diet. An additional group received L + 0.5 kg concentrate/head per day (LC). The concentrate consisted of barley, sugar beet pulp, citrus pulp, soyabean meal, molasses and mineral vitamins at 350, 175, 175, 250, 25 and 25 kg/t, respectively. The four treatments were offered to 248 spring born ewe lambs (initial live weight 35.8 (s.d. 3.83) kg) in a fully randomised design and was analysed using Proc GLM of SAS. *A priori* contrasts were used to test the effects of concentrate and

the response of increasing herbage allowance. The ewe lambs were allocated to treatments balanced with respect to flock of origin. Pre- and post-grazing height and sward mass were recorded weekly for the duration of the study. The treatments, which were grazed as one replicate per treatment, were rotated weekly within the paddocks to reduce any effect of position of treatment within paddock on subsequent performance. The ewe hoggets were grazed as one flock from 4 March to 11 August to evaluate the effect of treatment on subsequent growth rate.

**Table 5: Effect of grass allowance and concentrate supplementation on food intake and animal performance**

Variable	Herbage allowance (HA;kg DM/d)				s.e.	Significance		
	0.75	0.75+C <sup>§</sup>	1.25	1.75		HA Linear	HA Quad	Conc v no conc
Grass intake (kg DM/d)	0.61	0.57	0.92	1.24	0.06	***	NS	NS
Grass utilisation (g/kg)	822	727	733	684	30.7	**	NS	*
Live weight (kg) at end of:								
extended grazing	35.8	42.2	39.8	42.3	0.32	***	*	***
grazing season	52.5	55.8	53.4	56.2	0.64	***	NS	***
Live-weight change (g/d) during:								
extended grazing	-1.0	84	52	84	4.2	***	P=0.06	***
grazing season	103	83	85	88	4.0	***	*	***
Total study	69	83	73	85	2.7	***	NS	***
Condition score at end of :								
extended grazing	2.77	3.18	2.91	3.03	0.034	***	NS	***
grazing season	3.17	3.25	3.16	3.30	0.055	NS	NS	NS

<sup>§</sup>Plus 0.5 kg concentrate per head daily.

The swards offered had mean herbage DM mass, height and concentrations of DM and crude protein of 2332 kg/ha, 8.15 cm, 204 g/kg and 200 g/kg DM, respectively. The effects of herbage allowance and concentrate supplementation on herbage intake and animal performance are presented in Table 5. Increasing grass allowance increased herbage intake, live weight at the end of extended grazing and of the grazing season (mid March to mid August), live-weight gain during extended grazing and total experimental period and condition score at the end of extended grazing. Increasing herbage allowance reduced herbage utilisation during extended grazing and daily live-weight gain during the grazing season but did not alter condition score at the end of the grazing season. Concentrate supplementation increased live weight at the end of extended grazing and grazing season, live-weight gain during extended grazing and overall and condition score during extended grazing. Concentrate supplementation reduced herbage utilisation but did not alter herbage intake or final condition score. The potential herbage sparing effect due to 0.5 kg concentrate daily during extended grazing, as determined by live-weight gain during extended grazing and the total experiment was 1.0 and 0.95 kg DM, respectively. Relative to treatment H, compensatory growth for treatments L and M was 0.43 and 0.0, respectively.

It is concluded that increasing herbage allowance during extended grazing increased herbage intake and animal performance, and decreased herbage utilisation. Supplementing with 0.5 kg concentrate replaced 1 kg herbage DM of extended grazing herbage allowance. Compensatory growth during the grazing season did not remove the effect of extended grazing treatments on animal live weight.

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### **Effect of maturity of maize at harvest and system of ensiling grass silage on ewe performance and subsequent lamb growth rate**

A large proportion of the national ewe flock is housed during the winter feeding period and offered predominantly grass silage based diets. Forage quality offered during the housing period is the third most important factor (after ewe genotype and grassland management) affecting the cost of producing lamb in mid season flocks. In early-lambing flocks winter forage quality has a greater impact on the costs of lamb production. Recent costings have illustrated that in a three-cut silage system big bale silage can be produced and fed to livestock at a similar cost to precision chop silage. Whilst it is estimated that 55% of silage on sheep farms is ensiled in big bales there is a paucity of research data to illustrate the impact of the big bale silage system on ewe and subsequent lamb performance. Furthermore possible interactions between ensiling system and silage feed value need to be evaluated to determine the impact on concentrate supplementation requirement.

Maize silage is increasing in popularity on beef and dairy farms. The yield potential of maize has increased by 300% in the last 30 years due essentially to improvements in plant breeding. The use of the complete cover plastic mulch system has further increased yield potential by up to 6 t DM/ha. Whilst numerous studies have shown the benefits of maize silage inclusion in the diet of beef and dairy cattle, few studies have been undertaken to evaluate the impact on ewe performance.

The objective of the present study is to evaluate the effects of maturity of maize and level of inclusion in the diet, feed value and system of harvest of grass silage on ewe performance and subsequent lamb growth rate. Furthermore the potential concentrate sparing effect of offering maize silage, high feed value grass silage and system of harvesting grass silage will also be determined.

The present study was established in the autumn and involves a total of 12 treatments consisting of two grass silage harvests (first and second) by two grass silage harvest systems (big baled, precision chopped) by two concentrate feed levels (low, high) plus two maize silages (low DM, high DM), by two levels of protein nutrition.

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### **Effect of season of shearing on ewe productivity and subsequent lamb performance**

The number of lambs weaned per ewe is the major factor influencing profitability for lamb production. Currently there is interest within the sheep industry to shearing ewes pre mating. Whilst some studies in Nordic countries and New Zealand have reported benefits to shearing pre mating on the fertility of ewes lamb and 2-tooth ewes, there is no such evidence from temperate climates similar to Ireland. The response to shearing pre-mating on ewe fertility may be related to ewe age, breed, condition score and weight. The objective of the present study is to evaluate the effects of season of shearing on ewe fertility and productivity and on subsequent lamb birth weight and growth rate.

Post implementation of the Mid Term Review of the Common Agricultural Policy it is essential to improve efficiency and reduce cost of production if sheep production is to remain a viable farm enterprise in the absence of subsidy. For animal welfare reasons ewes must be shorn at least once per year. Recent studies at Athenry have reported that shearing ewes at housing increased lamb birth weight and subsequent weaning weight by 0.6 and 2.4 kg, respectively. This improvement in performance enables lamb to be marketed two weeks earlier at a higher price per unit carcass weight.

The current study involves 130 ewes allocated to 4 treatments consisting of four shearing regimes as follows, shorn either prior to mating, at housing, in June or both in June and prior

to mating. This study was initiated in mid 2006 and preliminary results will be available in 2007.

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### **An on-farm evaluation of the effect of season of shearing on ewe productivity and subsequent lamb performance**

Sheep are shorn at least once per year for animal welfare reasons. Previous studies at Athenry have shown that season of shearing impacts on lamb birth and weaning weights and subsequent age at slaughter. Furthermore greater numbers of animals are available and potential interactions with ewe characteristics can be evaluated.

The objective of the current study is to evaluate the effects of season of shearing on commercial farms and potential interactions with ewe characteristics (age, breed, weight, condition score) on ewe and subsequent lamb performance. This study involves approximately 1000 ewes and their progeny on commercial farms shorn at different stages during the annual production cycle.

*Keady, T.W.J. and Hanrahan, J.P.*

*RMIS No. 5674*

### **An evaluation of the effect of plane of nutrition at different phases during the rearing phase of replacement ewes, differing in genotype, on lifetime performance**

One of the major expenses in sheep production is replacement cost. In flocks there is approximately 22% replacement rate due to ewe loss either to health issues or old age. The number of lambs weaned per ewe annually, but ultimately during her lifetime, is the major factor effecting profitability from sheep production.

A previous study at Athenry reported that increasing grass dry matter allowance during the first winter extended grazing from 0.75 to 1.75 kg/day produced ewe replacements which weighed 35.8 and 42.3 kg at the end of extended grazing and 52.5 and 56.2 kg at the end of the second grazing season. This study illustrated that replacements can be reared using different regimes to 1.5 years of age, however, there is a paucity of data on the impact of plane of nutrition at different phases during the rearing regime of replacements on subsequent ewe productivity and longevity. The objective of the current study is to evaluate the effects of plane of nutrition at different phases during the rearing regime of replacement ewes, differing in genotype on subsequent lifetime performance.

The current study was initiated in autumn 2006 and involves 8 treatments consisting of two planes of nutrition in the first winter period by two planes of nutrition in the second grazing season, offered to 90 ewe lambs of two genotypes.

*Keady, T.W.J. and Hanrahan, J.P.*

*RMIS No. 5682*

### **The effects of grazing management, date of closure and subsequent harvest date on herbage yield and feed value**

Previous studies at Athenry have reported that extended grazing ewes in mid, late or throughout pregnancy increased lamb birth and weaning weights relative to lambs from ewes which were housed and unshorn (Research Report 2005, pp112-113). Furthermore previous studies have shown that during extended grazing herbage utilisation rates are low, depending on herbage allowance and unaffected by period of residency in the paddock. A previous study at Athenry also clearly reported that each 1 day delay in grazing after early December reduced herbage yield the following spring by 52 kg DM per day delay in grazing. The aim

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of this study was to further evaluate the effect of extended grazing management on herbage yield and feed value during the subsequent grazing season. The current study involves a total of 64 treatments consisting of four dates of extended grazing (ranging from December to January 31) by two herbage allowance at grazing by two residency periods by four harvest dates. Each treatment was replicated twice. Results will become available during 2007.

*Keady, T.W.J. and Hanrahan, J.P.*

*RMIS No. 5682*

### **Organic system for sheep at Mellows**

The sheep flock in the organic farm is managed as a self-contained sheep-only system but the area assigned each year has not been grazed by sheep in the previous year. The land block assigned for 2006 season comprised 6 paddocks (total area 16.6 ha). A key objective of the grazing plan is to minimise the intensity of parasite challenge experienced by the lambs so that they can be finished without the need for anthelmintic treatment. After weaning in 2006 the lambs were given access to silage aftermath outside the main grazing area and had essentially *ad libitum* access during July and August.

A total of 123 ewes were joined with rams in autumn 2005. The mating plan involved use of Belclare rams on a proportion of the flock to produce prolific replacement ewes while the remainder of the flock was joined with 2 Texel rams. Texel rams were used in preference to Suffolk because research at Athenry has shown that the Texel breed has a much greater resistance to parasites and this difference is reflected in crossbred progeny.

A total of 109 ewes were recorded as lambed (mean lambing date 9 March) and average litter size was 1.93. From a 210 lambs born, 194 were alive and tagged at birth while the number of these that survived to 5-weeks of age was 174 (10% mortality relative to live births). Mean birth weights were 5.4, 4.5 and 3.4 for singles, twins and triplets, respectively. Lamb growth rate from birth to the 26 April averaged 254 g/day. The growth performance from the end of April to 1 June averaged 317 g/day while the growth rate from 1 June to 27 June averaged 272 g/day. Lambs were weaned at the end of June. The overall growth rate from birth to weaning was 272 g/day. The breed of ram used had no significant effect on lamb growth rate. Post-weaning growth rate was quite satisfactory; the average growth rate between weaning and end of July was 194 g/day while for the next month (to end of August) the daily gain was 190 g. Lambs were drafted for slaughter as follows: 14 on 3 July, 33 on 25 July, 65 on 1 September, 21 on 11 October and 10 on 11 November. Average carcass weight was between 20 and 21 kg. A total of 33 ewe lambs were retained as replacements.

Roundworm parasite levels were monitored weekly in ewes, lambs and hoggets during the 2006 grazing season. Faecal egg counts (FEC) were determined using the FECPAK<sup>®</sup> method from pooled faecal material (at least 10 animals). To prevent problems due to roundworms the flock graze 'clean' pastures at turnout (i.e. not grazed by sheep in the previous season) and ewes were dosed at turnout to reduce pasture contamination and replacement hoggets were dosed prior to entering the grazing area. The pattern of FEC values for the ewes and lambs and are shown in Figures 1 and 2. With the exception of a peak on the 21 June the FEC values for lambs remained near or below 500 eggs per gram for most of the season. The overall general decline observed between June/July and October/November may be attributed to the lambs grazing aftermath which would have lower parasite challenge. The FEC values in the replacement hoggets were negligible since turnout in spring (data not shown).

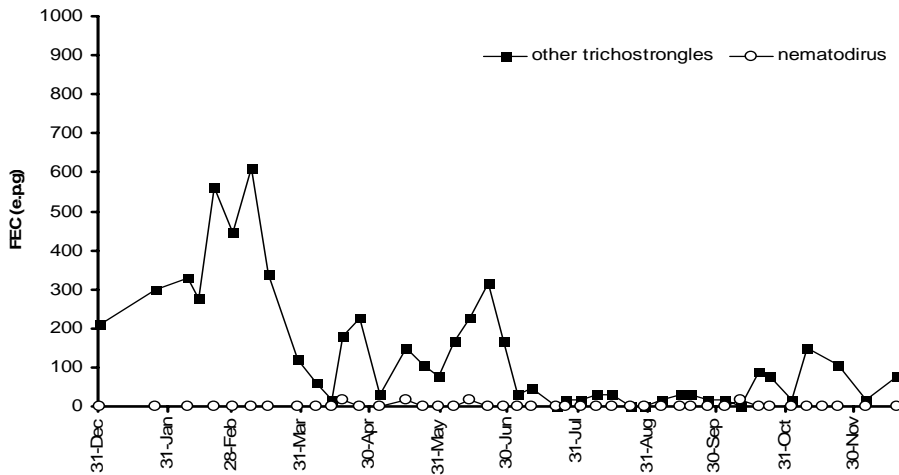


Figure 1. FEC for ewes in the organic flock in 2006.

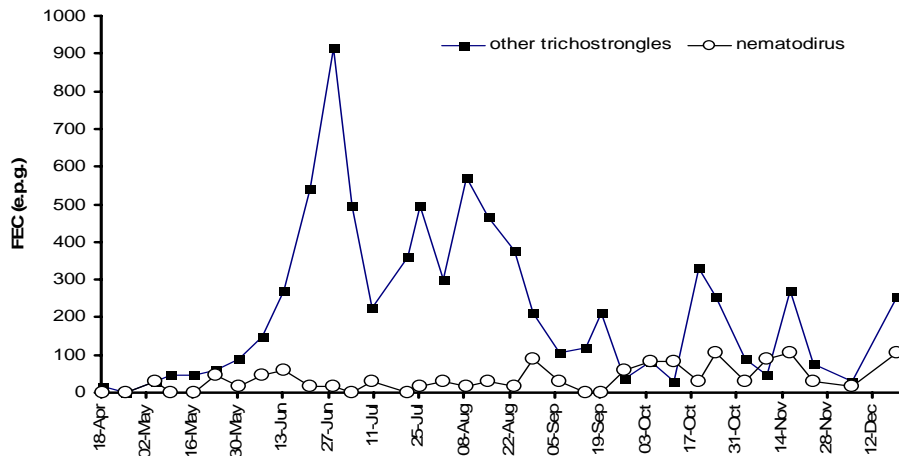


Figure 2. FEC for lambs in the organic flock in 2006.

Hanrahan, J.P, Good, B. and Nally, J.

RMIS No. 5253

**Hill Sheep Farm: flock performance details for 2006**

The management system consisted of a Hill flock (2 to 4 year old ewes only) and a Lowland flock (ewes older than 4 years plus a small number of 2 year old ewes). Ewes in the Hill flock were joined with purebred Scottish Blackface rams in single-sire groups but two of the 5 groups were also exposed to Chamois rams for about 10 days and then the assigned Scottish Blackface rams. The Lowland flock ewes were joined with either Belclare (n=2) or Chamois (n=2) rams in single sire groups.

The management procedures were essentially the same as in previous years. Thus, single-born female lambs in the Hill flock were put of the hill at about 5 weeks of age. The remaining Hill-flock lambs were randomly assigned to either a creep-concentrate or no-creep groups and these lambs remained on the greenland. In the case of the Lowland flock, lambs were assigned at random to either a creep or non-creep group at 5 weeks of age. Creep was allocated up to a maximum of 300 g per lamb per day and intake was monitored by recording cumulative intake at about weekly intervals. Creep feed assignments were continued up to 16 October 2006. At this stage all lambs that were destined for slaughter were housed and creep was offered *ad libitum*. Lambs were housed in four groups that corresponded to the

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combinations of creep treatment up to 16 Oct by flock (Hill or Lowland). Lambs were weighed at birth and at 5, 10, and 14 weeks of age (except single born S. Blackface female lambs were not weighed at 10 weeks). All lambs on the farm were weighed in late September, on 16 October and on 20 November.

The information on ewe reproductive performance and lamb mortality is summarised in Table 6. The incidence of barren ewes was higher than usual. There was no evident association with mating group so no obvious explanation can be offered. The total mortality incidences for single lambs were 3.0% and 3.3% for the Hill and Lowland flocks, respectively. The corresponding value for twins in the Lowland flock was 6.4% and thus litter size differences explain the higher lamb mortality in the Lowland system.

**Table 6: Ewe performance details for hill sheep flock - 2006**

Variable	Flock	
	Hill	Lowland
No. Ewes joined	201	130
No. Ewes barren	13	9
No. Ewes lambed	185	119
Ewe mortality <sup>§</sup> (%)	3.0	3.1
Litter size	1.09	1.53
No. lambs reared		
-per ewe joined	0.99	1.35
-per ewe lambed	1.06	1.45
Lamb mortality (%)		
- At birth	2.0	3.3
- Total to 5 weeks of age	3.0	4.9

<sup>§</sup> In each flock the mortality includes one ewe declared as dead but was actually missing.

**Table 7: Lamb growth in Hill and Lowland systems - 2006**

Variable	Category	Flock	
		Hill	Lowland
Birth weight (kg)	Single	4.0 ± 0.04	4.7 ± 0.09
	Twin	3.3 ± 0.10	3.6 ± 0.10
Growth rate (g/day)			
	-0 to 5 weeks		
	Single	266 ± 4.1	334 ± 6.7
	Twin	198 ± 8.8	269 ± 7.3
-5 to 14 weeks			
	Single-female	146 ± 6.5	-
	Single-male	203 ± 3.9	-
	Single	-	221 ± 5.2
	Twin	-	211 ± 5.3
-0 to 14 weeks			
	Single-female	178 ± 6.0	-
	Single-male	221 ± 3.7	-
	Single	-	255 ± 5.0
	Twin	-	229 ± 5.4
Weight (kg) at 14 weeks	Single-female	21.4 ± 0.62	-
	Single-male	25.7 ± 0.38	-
	Single	-	29.7 ± 0.53
	Twin	-	26.2 ± 0.56

The least squares means for lamb growth traits are summarised in Table 7. Note that in the case of the Hill flock there are very few twin-born lambs and that single-born lambs are put to the hill grazing at 5 weeks of age. Accordingly, means are given for single-female or single-male categories for the Hill flock for growth periods that extend beyond 5 weeks of age. The large difference between male and female singles in the Hill flock for growth past 5 weeks is attributed to the different vegetation types available to the ewes. In the Lowland flock the

difference between males and females is in the order of 19 g/day for growth rate to weaning- compared with the difference of over twice this magnitude in the Hill flock singles. The general pattern of change in lamb growth with age that is evident in Table 7, i.e. a large decline when the 0 to 5 week interval is compared with the 5 to 14 week interval, is consistent with that documented over 10 years for lowland systems at Athenry and Knockbeg. Likewise the effects of birth type in the Lowland flock are consistent with those recorded in Athenry.

Data on response to supplementary concentrates were examined at a number of stages. The first stage was the weight at weaning (14 weeks). The results of these analyses are in Table 8. In the case of the hill flock lambs the analysis was confined to lambs that were on the Greenland from birth (i.e. all male lambs and female lambs that were born as twins). The model for this analysis had effect for sex x rearing-type in the case of hill flock.

**Table 8: Effect of creep feed on weight (kg) at 14 weeks of age and growth rate**

Variable	Treatment	Flock	
		Hill	Lowland
Weight at 14 weeks (kg)	Creep	23.9 ± 0.54	28.1 ± 0.48
	No Creep	23.1 ± 0.54	27.7 ± 0.50
	F test	p> 0.19	p> 0.5
Growth rate 5 to 10 weeks (g/day)	Creep	218 ± 5.8	258 ± 6.2
	No Creep	212 ± 5.8	239 ± 6.4
	F test	p> 0.3	P< 0.01
Growth rate 5 to 14 weeks (g/day)	Creep	199 ± 5.8	216 ± 4.8
	No Creep	191 ± 5.8	217 ± 5.0
	F test	p> 0.19	p> 0.8
Growth rate 10 to 14 weeks (g/day)	Creep	178 ± 9.5	169 ± 6.8
	No Creep	165 ± 9.4	194 ± 7.1
	F test	p>0.2	P< 0.01
Growth weaning to late August (g/day)	Creep	90 ± 9.9	105 ± 6.0
	No creep	65 ± 10.1	79 ± 6.0
	F test	P<0.05	P<0.001

The response to creep feeding was not statistically significant in either flock for weight at weaning. In the lowland flock the lambs on creep feeding had a significantly lower growth between 10 and 14 weeks of age than the lambs without access to creep and there was no evidence for any interaction between creep effect and rearing type for this variable. Both hill-flock and lowland-flock lambs on creep feed had significantly higher growth rate between weaning and late August than corresponding lambs without access to creep feed.

Lambs (females) that were selected for breeding for either Leenane or Athenry were removed from the supplementary creep regime in September. In order to assess the longer-term effects of creep feed on lamb weight those lambs that were destined for slaughter in October to December period were examined as a separate subset of data. The impact of creep feed on live weight on 25 September and 16 October are summarized in Table 9. The number of lambs with weights in September, October and November were 86, 60 and 38, respectively, for the Lowland flock; the corresponding numbers were 91, 45 and 32 for Hill flock.

The effect of creep feed on weight in September was significant in the Lowland flock but while the Hill flock lambs were 1.1 kg heavier in September this difference was not

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statistically significant. The subsequent weights are difficult to interpret as the lambs were drafted for sale between September and November based on live weight.

**Table 9: Effect of creep feed on live weight – in September to November period**

Variable	Treatment	Number of lambs <sup>§</sup>	Flock	
			Hill	Lowland
Sep weight (kg)	Creep	44, 40	30.7 ± 0.61	37.7 ± 0.88
	No Creep	41, 46	29.6 ± 0.61	35.4 ± 0.91
			P > 0.2	P < 0.05
Oct weight (kg)	Creep	16, 27	27.5 ± 0.78	36.2 ± 0.83
	No Creep	23, 33	28.6 ± 0.60	34.6 ± 0.87
			P > 0.2	P = 0.11
Nov weight (kg)	Creep	16, 20	29.2 ± 0.98	36.3 ± 1.39
	No Creep	10, 18	28.5 ± 1.08	36.0 ± 1.18

<sup>§</sup> The number in hill and lowland flocks, respectively.

Between 16 October and 15 November a set of 68 lambs were housed and offered creep feed *ad libitum*. These lambs represented 4 prior management groups and the performance is summarised in Table 10. Groups did not differ in growth performance for the period up to Nov 20 either with respect to prior feeding regime or source (Hill or Lowland). However the growth rate during the period was extremely low (29 g/day for the Hill groups and 80 g/day for the Lowland lambs). The daily food consumption over this indoor period averaged 1.08 kg and 0.85 kg for Lowland and Hill lambs, respectively.

**Table 10: Growth performance indoors**

Experimental Group	Live weight (kg)		
	Oct 16	Nov 20 <sup>§</sup>	No. of lambs
Hill-creep	26.0	32.2	19
Hill- No creep	24.8	32.7	13
Lowland-creep	33.1	33.9	14
Lowland- No creep	33.6	33.6	24

<sup>§</sup>Adjusted for variation in weight on 16 October.

Thus, the feed conversion ratios were 13:1 and 28:1 for Lowland and Hill, respectively. It is evident that the gain achieved would not cover the cost in the use of the Hill lambs but may be close to cost recovery in the case of the Lowland lambs. Based on all of the evidence from impact of creep feed in 2006 it is clear that returns in terms of extra animal performances do not justify the costs involved. This evidence is consistent with estimated obtained previously for hill lambs.

*O'Malley, L. and Hanrahan, J.P.*

*RMIS No. 5080*

### **Habitat selection of hill sheep on the Teagasc hill sheep farm, using satellite tracking and field observations**

Upland and peatland in western Ireland are particularly susceptible to erosion and are typically dominated by the habitats, blanket bog and wet heath. Both habitats are designated as Annex I-listed under the EU Habitats Directive and are thus recognised as important for conservation.

The 255 ha Teagasc Hill Sheep Farm in Co. Mayo was chosen as the study site. The site consists of upland and peatland and the current annual stocking rate on the hill is 0.8 ewes per hectare (was 0.9 ewes per hectare prior to 1998). Habitat mapping was carried out using the Irish classification and following guidelines published by the Heritage Council. Twenty habitat types occurred on the study site with blanket bog and wet heath occupying 53% and

35%, respectively, of the 217 ha available to hill sheep. Nine habitats were available to sheep and suitable for habitat selection analysis.

Randomly selected Scottish Blackface ewes were tracked using Lotek 2200 global positioning system collars for 5-week seasonal sampling periods between 2004 and 2006. Ewe ranges and habitat use were analysed using Ranges7. Habitat selection analysis, comparing proportions of used habitat with those available, was performed using Compos Analysis v6.2+ which uses Wilks' lambda test (MANOVA). Habitat selection analyses were carried out at (i) a broad selection level comparing habitats present within ewe ranges with those available across the study area accessible to ewes, and (ii) a detailed selection level comparing habitats used at location with those available within ewe ranges.

**Table 11: Ranked habitat selection for 10 ewes tracked in spring 2004, 2005 or 2006 at the broad selection level**

Selection rank <sup>§</sup>	Habitat	Percent available
8	Wet heath	35.3
7	Acid grassland	3.0
6	Wet heath-acid grassland	2.5
5	Blanket bog	53.0
4	Dense bracken	1.5
3	Buildings & artificial surfaces (track)	0.0
2	Wet grassland	0.5
1	Blanket bog-acid grassland	1.5
0	Cutover bog	2.0

<sup>§</sup> Rank 8-0 represents habitats used most to those least used.

Habitat selection analyses indicated that there was a significant difference in habitat selection at the broad level ( $\Lambda = 0.0000$ ,  $P = 0.002$  by randomisation) but not at the detailed level ( $\Lambda = 0.0000$ ,  $P = 0.308$  by randomisation) suggesting that ewe ranges were based on habitat selection but habitat use within ranges was more uniform. Habitat selection rankings are presented in Table 11. Wet heath and acid grassland were selected most and cutover bog was selected least.

Uneven sheep distribution and habitat use across the study site indicate that monitoring grazing impact on upland and peatland habitats is essential. Knowledge of hill sheep behaviour obtained from this study, combined with grazing impact assessments, would enable more-informed management decisions. Findings suggest that of the two Annex I-listed, dominant habitats, grazing-related damage is less likely to occur on blanket bog and most likely to occur on wet heath when both habitats are available to grazers. Where necessary, measures to redistribute grazing pressure, such as seasonal grazing, temporary fencing or the use of supplementary feed blocks, should be considered.

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*RMIS No. 5080*

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**The impact of hill sheep activity on the population dynamics and diet of small mammals**

The project is based on the Teagasc Hill Sheep Farm, and the background is outlined in *Research Report 2005, p119*. The overall aim is to integrate biodiversity-promoting strategies and general agricultural practices. The objective is to assess the impact of hill sheep activity on the diet and populations of small mammals.

Wood mice were trapped from May to October 2006 using Longworth live traps. Sites were selected on the basis of sheep frequency observations. Twenty sampling sites were identified

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on the farm; 10 in areas with the highest frequency of sheep occupation and 10 in areas where sheep occupation frequencies were at their lowest. Wet heath was the dominant habitat in 14 sites, blanket bog in 5 and dense bracken in one. Each trapping session involved the use of 50 Longworth traps at each site over 4 nights. Captured wood mice were tagged with PIT tags and weight, sex, hind foot length, age group and breeding condition were recorded before immediate release at the point of capture. Details are summarised in Table 12. Wet heath was the dominant habitat in 70% of the sites in which 93% of the captures occurred.

**Table 12: Total captures, individuals captured and mean weight of individuals in areas of high and low frequency of sheep occupation**

Sheep frequency	Number of captures	Individuals captured	Mean weight (g)
High	76	24	16.3
Low	37	17	16.1

A significantly higher number of total captures but not of individuals occurred in the area of high frequency of hill sheep occupation. The weight of the wood mouse, which is an indicator of its fitness, was not affected by sheep frequency. The wood mouse tended to concentrate on the relatively dry, wet heath habitats and appeared to have a shorter breeding season than in woodland habitat.

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RMIS No. 5382

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### **Sustainable agri-environmental management of hill and mountain peatland**

The project is part of a large, EPA-funded programme (outlined in *Research Report 2005, p.119-120*) to achieve sustainable agri-environmental management of hill and mountain peat land. The objective of this study is to quantify the physical impact of hill sheep at low, medium and high levels of grazing by quantifying changes in micro soil erosion, plant cover and soil nutrient content over time.

Sites representing three grazing intensities (low, medium, and high) were selected in the Connemara region of Galway and Mayo based on altitude and physiography. Within each site four areas associated with sheep activity, namely high and low densities of occupation, sheep 'rests' and movement corridors, were chosen. These were identified largely by random selection from existing databases for the medium level of grazing and by field examination of physiographic transects in the low and high levels of grazing. Purple moor-grass (*Molinia caerulea*) is the dominant species at both levels of grazing in the Teagasc Hill Sheep Farm, Leenaun. Its frequency, however, increased by over 8 percentage points in areas of low activity compared with almost 5 percentage points in areas of high activity in the period 1995-2004.

Methods to measure changes in micro-erosion and deformation of soil surface include rainfall detachment trays (constructed), Gerlough troughs, splash cups, micro-topographic pin profilers (designed) and reference marker pins. All the sites have been sampled for soil fertility analysis. There were no definite patterns in the initial results.

Results of the micro-soil erosion, plant cover and soil nutrient analysis will be correlated with relevant farm management systems details and local weather data to identify sustainable agri-environmental management strategies of hill and mountain peatland.

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RMIS No. 5547

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**Pedigree Galway sheep – population size and performance details**

All flocks registered with the Galway Sheep Breeders Association Ltd. and located in the Republic of Ireland participate in the Galway Sheep Breed Improvement programme operated by the Department of Agriculture, Food and Rural Development. This programme involves the recording of litter size data on individual pedigree Galway ewes with the objective of calculating a merit index for prolificacy for individual ewes and their progeny. Breeders are encouraged to use this information in the selection of breeding stock so as to bring about genetic improvement in prolificacy. The data collected under the programme are entered, validated and processed by the Sheep Production Department at Athenry.

A total of 670 ewes, representing 42 flocks, were recorded as having produced purebred Galway lambs and this included 16 that were joined as ewe lambs. Only 75% of the ewes listed on the original mating plan were recorded as having lambed to a purebred mating. The number of pedigree ewes and flocks participating in the programme is now similar to the peak values in 2000. A total of 56 rams, from 20 different flocks, sired progeny in 2006. Thirteen of these rams were born in 2005 while 22 were born in 2004. These numbers indicate that the genetic base of the breed and the effective size of the population are increasing at present. The proportion of the new flocks that persist with the Galway breed and the continued presence of the core of long-established Galway flocks are both vital to the long-term prospects for conservation of the breed.

The litter size data for all ewes are summarised in Table 13 by age of ewe and the performance is consistent with results for recent years (*see Research Report 2005, p122-123*).

**Table 13: Litter size in 2006 for pedigree Galway ewes in 42 flocks**

<i>Ewe Age<sup>+</sup></i>	<i>No. of ewes</i>		<i>Litter size</i>	
	<i>Joined</i>	<i>Lambled</i>	<i>Total</i>	<i>Live</i>
1	57	16	1.00	0.94
2	208	161	1.54	1.32
3	230	191	1.69	1.51
4	171	143	1.62	1.53
5	227	163	1.60	1.44
<b>Overall</b>	<b>893</b>	<b>674</b>	<b>1.62</b>	<b>1.48</b>

<sup>+</sup> Age at lambing

Hanrahan, J.P. and Curley, A.

RMIS No. 5254

**Genetic diversity in Galway sheep compared with Suffolk and Texel breeds**

The Galway breed is the only native Irish sheep breed and is listed as a breed in danger of extinction. As part of the genetic conservation and overall study of the breed a DNA bank has been established. This bank is being used to characterise the genetic variability of the breed. The comparison of variability in Galway sheep with that in Texel and Suffolk breeds based on 16 microsatellite loci was completed (*see Research Report 2005, p 120-121*) and the results are summarised in Table 14. Suffolk displayed the lowest expected heterozygosity and also had the smallest number of alleles. Galway sheep yielded the highest value for both heterozygosity and number of alleles per locus.

**Table 14: Summary statistics for microsatellite markers in Galway, Texel and Suffolk sheep**

Population	Sample size	Loci typed	Observed heterozygosity		No. of alleles	
			Estimate	s.d.	Mean	s.d.
Galway	94	15	0.6037	0.0138	5.13	0.99
Suffolk	70	15	0.5344	0.0208	3.73	1.22
Texel	58	16	0.5249	0.0239	4.44	1.26

Samples of animals from each breed are being analysed for variation in mitochondrial DNA and for variation in the male specific region of the Y chromosome based on a single nucleotide polymorphism (oY1) and the microsatellite SRYM18.

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RMIS No. 5254

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### Conservation of semen from Galway rams

The programme to collect and conserve semen from a representative sample of Galway rams was continued (*see Research Report, 2005, p122*). For the autumn of 2006 a panel of 14 rams was assembled. This included rams purchased in previous years and that had not yielded an adequate number of straws of freezable quality plus a set of 6 new rams (1 purchased and 5 on loan). The details on the rams are in Table 15.

**Table 15: Panel of Galway rams assembled for semen collection in autumn 2006**

Ram tattoo <sup>1</sup>	Breeder	No. of straws frozen in 2006 season
FB/05/0075	Forde	151
FB/05/0091	Forde	190
FT/04/0349	Feeney	108
GD/04/0421	Daly	66
GD/05/0519	Daly	169
GO/00/3	Nolan	105
JC/02/046	Casey	85
JC/03/0304	Casey	0 (Sterile)
JM/03/0513	Maher	180
MM/03/0112	Murphy	25
MM/04/0129	Murphy	213
PS/03/0022	Sice	164
PS/04/0758	Sice	0 (Sterile)
TK/01/19	Keville	165

<sup>1</sup>Ram tattoo is as recorded on Galway pedigree file but with '/' inserted to set off flock code, year of birth and number.

The results show that no freezable semen was obtained from two rams that had not yielded semen in previous years while one other ram (JM/03/0513) performed satisfactorily and yielded 180 straws. The total number of straws collected in this programme is 3420 and this represents 19 rams. The original target was to obtain semen from 50 rams but due to the high failure rate and the difficulty in sourcing rams early enough in the breeding season to allow adequate time for training this target was not met. A total of 13 rams were purchased at annual sales in order to ensure that adequate quality semen and large numbers of straws from the rams available. When each year's ram panel is considered as independent 25% of rams failed to yield semen. This high failure rate was unexpected.

Donovan, A., Lally, T. and Hanrahan, J.P.

RMIS No. 5254

**Evaluating the impact of pedigree sheep breed improvement programme (PSBIP) for terminal sire breeds**

The background and context of this project were given in the Research Report 2005 (p126-127). Rams used either come from PSBIP flocks and thus have a lean meat index (LMI; rams with high index values are chosen) or they represent flocks that do not participate in the PSBIP and their sires did not come from PSBIP flocks. In the production year 2005/2006 four commercial flocks were used and 29 rams yielded progeny-test data (2 rams provided no progeny due to death or infertility). This number is lower than in the previous year because the sheep flock at Knockbeg was dispersed at the end of 2005. Of the set of rams tested 4(2 Charollais and 2 Suffolk) were also involved in the panel tested in 2005. This was done to increase the number of progeny for particular sires but also provided some linkage across years. Details on the panel of rams tested in 2006 are set out in Table 16. The mean LMI values were similar to those of the panel used for the 2005 test. The number of progeny per sire was somewhat lower especially in the case of the Charollais breed. However, the numbers are close to the target of 50 progeny per sire.

**Table 16: Pedigree rams tested in 2006 categorized by breed and LMI status**

Breed	LMI status	No. of rams	Mean LMI	Mean no. of progeny
Charollais	High	4	138	45
	None	7 <sup>§</sup>	-	51
Suffolk	High	9 <sup>§</sup>	157	63
	None	9 <sup>§</sup>	-	56

<sup>§</sup> Two were also represented in 2005 data set.

Mean values for selected lamb growth traits are given in Table 17 as an indication of the general level of performance at which the rams were tested.

**Table 17: Mean values for lamb growth traits in 2006**

Breed of sire <sup>§</sup>	Weight (kg) at			Growth rate(g/day)
	Birth	14 weeks	120 days	0 to 14 weeks
Charollais	4.9	30.7	35.2	263
Suffolk	5.0	33.1	38.3	286

<sup>§</sup> Note that breed is confounded with flock.

The association between LMI values and lamb weight at 14 weeks of age (W14A) is summarized for each breed in Figures 3 and 4. These show the individual sire effects on W14A; the corresponding LMI value is shown for each sire (a zero indicates rams from flocks that were not involved in the PSBIP).

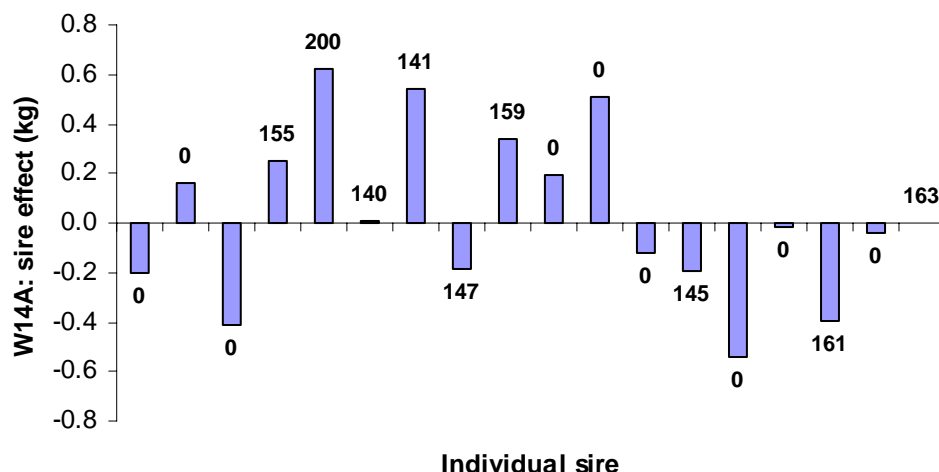


Figure 3. Effect of sire on weight at 14 weeks of age – Suffolk.

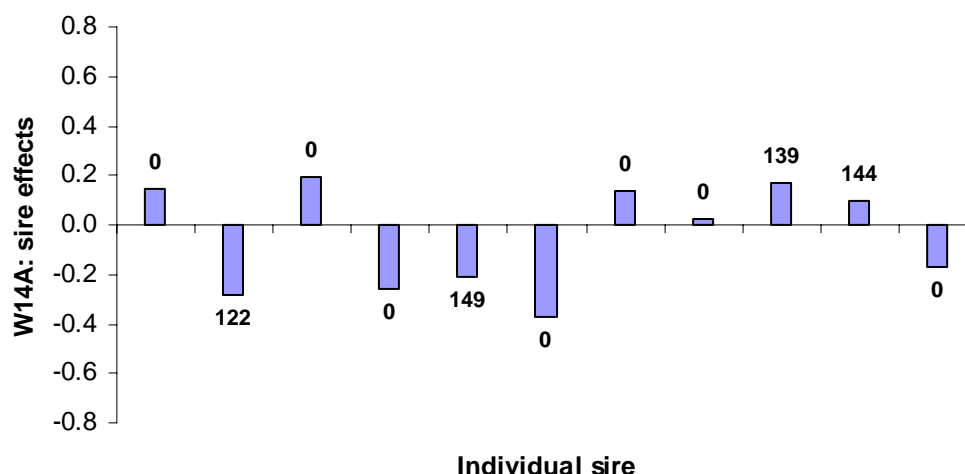


Figure 4. Effect of sire on weight at 14 weeks of age – Charollais.

The differences between LMI and non-LMI sires were estimated and were not statistically significant for any growth trait in either breed. The estimated difference (LMI minus non-LMI) for W14A was -0.02 (s.e. 0.284) kg for Charollais and 0.15 (s.e. 0.207) kg for the Suffolk breed. These differences are very small and are consistent with the results from 2005. The weighted average across years for the effect on W14A was 0.02 (s.e. 0.186) kg for Charollais and 0.17 (s.e. 0.150) kg for the Suffolk breed. The results suggest an effect of LMI status on birth weight in that positive differences were obtained for both breeds in 2005 and 2006. The weighted average difference across breeds and years was 0.051 (s.e. 0.0243) kg and this difference was significant ( $P < 0.05$ ). This suggests that the genetic correlation between LMI and birth weight is positive and this is consistent with the changes observed in Suffolk and Texel lines at Athenry that have been selected for pre-weaning growth rate.

Hanrahan, J.P.

RMIS No. 5389

#### Association between ewe breed type and longevity in a lowland production system

The length of productive live is an aspect of ewe performance evaluation that has received very little attention. Longevity estimates for four breed types in a lowland system (over a 5-year period) were reported in the late 1960s (see Animal production Research Report 1967,

p73-75). That study involved purebred (Galway, Cheviot) and crossbred (Halfbred, Greyface) ewes and while purebred ewes had the lowest longevity (4.25 & 4.3 v. 4.44 & 4.66 years) they also had significantly lower prolificacy. This apparent relationship is surprising. However, the study involved a small number of ewes. Belclare-X ewes rear significantly more lambs than a range of crossbred types (see Research Reports, 1994, p 37 & 1996 p22-23) but the impact of this increased production on longevity in lowland production systems has not been examined. The objective was to examine the difference in ewe longevity between two breed types that differ in prolificacy, and to assess associated effects on the main reasons for ewe disposal under defined consistent management conditions. The information used was extracted from ewe performance records for the flock at the Teagasc sheep unit at Knockbeg, Co. Carlow for the period 1988 to 2005. For most of this period the flock was divided between mid-season and early-lamb systems and was managed by the same person throughout. After the establishment phase most ewe replacements entered the farm as 2-tooths, although older ewes were introduced into the early-lamb system in some years, and generally remained on the farm until deemed unsuitable for further breeding. Replacement 2-tooth ewes were normally recruited into the mid-season system and were transferred to the early-lamb flock as they aged. All deaths were recorded and ewes for culling were removed in late summer. Reasons for culling were recorded as mouth (teeth broken, lost, excessive wear), udder (mastitis or other faults), poor condition (deemed unlikely to reach adequate

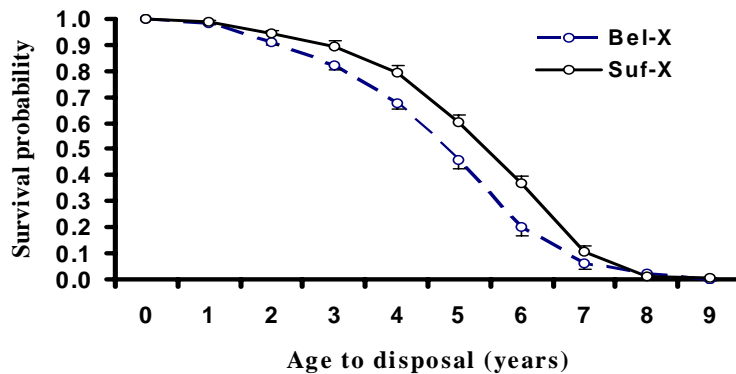


Figure 5. Survival probability curves for two breed types.

body condition by start of next breeding season), vaginal prolapse (recorded at lambing). Barren ewes were not culled except in a few cases. In certain circumstances ewes were culled although suitable for further breeding, due to adjustments of overall flock. Information on a set of Belclare-X-Cheviot (n = 357) and Suffolk-X-Cheviot (n = 268) ewes born between 1986 and 1995 was used for the present study. These ewes were produced contemporaneously from Cheviot dams and while their pattern of residency in the mid-season and early-lamb flocks was not controlled the differences are likely to have been relatively minor although Suffolk-X ewes may have been preferred in the early-lamb system. Data were analysed using SAS procedures (LIFETEST for survival probability, GENMOD, with a logit link function, for the incidence of various culling reasons).

The survival probability curves for the two breed types are shown in Figure 5. The curves were significantly different ( $P < 0.01$ ) and the associated average longevity estimates were: 5.1 (s.e. 0.09) and 5.7 (s.e. 0.10) years for Belclare-X and Suffolk-X ewes, respectively. These values are considerably greater than those reported previously (1969) but this is at least partly due to differences between the studies in culling policy. The breed effect was also significant when survival was based on time to death or culling for udder defects with censoring. This is not unexpected given the higher litter size and number of lambs reared for Belclare-X ewes. Results on ewe breed differences in the incidence of the various disposal reasons are presented in Table 18.

## Athenry Research Centre

**Table 18: Contribution (%) of disposal categories for two breed types**

Disposal reason	Ewe breed		Significance
	Belclare-X	Suffolk-X	
Died	14.8	10.8	P=0.14
Udder	25.2	20.5	P=0.17
Mouth	31.9	35.1	P=0.41
Poor condition	19.0	17.9	P=0.35
Vaginal prolapse	1.7	5.2	P<0.02
Miscellaneous	7.8	7.5	P>0.9

The breed-specific survival probabilities (Figure 5) translate into annual replacement rates of 23% and 21% for Belclare-X and Suffolk-X, respectively. These estimates are close to the value of 20% reported for a high output sheep system at Athenry (Nolan and McNamara, 2002). The annual replacement rate for Belclare-X ewes is 1.09 times that for Suffolk-X ewes and a difference of this order would add about €1.2 to annual ewe replacement cost.

Further evidence on the association between prolificacy and longevity is required and if the present findings are confirmed this will have to be included in assessments of the financial benefits of higher ewe productivity.

*Hanrahan, J.P.*

*RMIS No. 4785*

### Performance of pedigree Belclare sheep flocks

A total of 31 pedigree flocks recorded performance data for 2005/6 season. Total ewes with litter size information was 834 and weights on 1323 lambs were also recorded. Ewes older than 5 years at lambing were classified as 5 years and the performance, by ewe age, is summarised in Table 19. The results are consistent with previous years (see Research Reports for 2004, p 157 and 2005, p 128). The average litter size for all ewes, excluding ewe lambs was 2.14, and ranged from 1.68 to 2.59.

**Table 19: Reproductive performance of pedigree Belclare sheep**

Ewe age	No. of ewes	Litter size	
		Total born	Live born
1	96	1.65 ± 0.089	1.10 ± 0.097
2	311	1.99 ± 0.050	1.64 ± 0.054
3	213	2.21 ± 0.060	1.75 ± 0.065
4	98	2.28 ± 0.082	1.91 ± 0.089
5+	116	2.09 ± 0.077	1.78 ± 0.084

The incidence of lamb mortality was considerably higher than in previous years. Thus, for 2004 and 2005 the mortality for single and twin births was 4.1 and 11.1%, and 9.1 and 11.4%, respectively. The corresponding values for 2006, excluding ewe lambs, which were not represented in the previous years, were 8.2% and 16.5%. There is no obvious explanation for the major increase recorded in 2006. There was a very poor relationship between lamb mortality and average litter size and inspection of the flock means suggested that in 7 of the 29 flocks there was an excessive level of mortality and some of these were flocks with an average litter size less than 1.9.

Results for lamb growth are based on 1323 birth weight records and 1165 growth rate values. The birth weight and growth performance were similar to those reported for recent years. The growth rate recorded for progeny of yearling ewes was about 40 g/day lower than the mean values in Table 20.

**Table 20: Lamb growth rate to about 8 weeks of age**

Birth type	No. of records	Birth weight (kg)	Growth rate (g/day)	Age interval (days)
1	149	4.3	342	55
2	598	3.8	301	56
3	317	3.4	270	56
≥4	110	3.0	265	60

Hanrahan, J.P. and Curley, A.

RMIS No. 4785

**Effects of BMP15 and GDF9 mutations on ovarian follicle population and gene expression in Cambridge ewes**

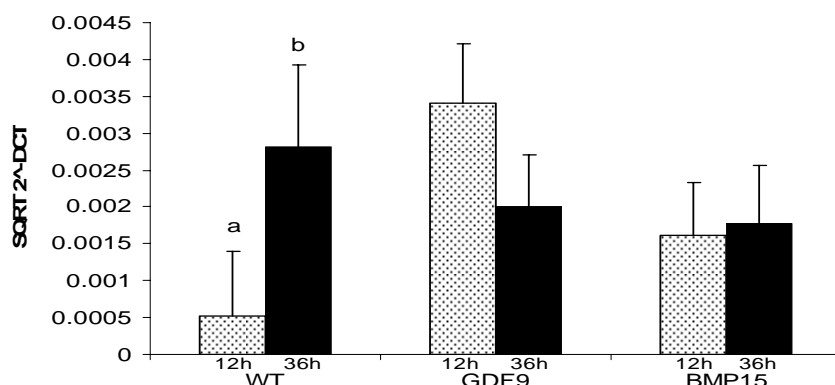
The high ovulation rate of the Cambridge breed is due to the presence of carriers of mutations in BMP15 and GDF9 genes (see Research Report 2004, p154-155). These mutations (*FecX<sup>G</sup>* and *FecG<sup>H</sup>*, respectively) increase ovulation rate in heterozygous carriers by 1.18 and 2.35 ova, respectively. BMP15 and BDF9 code for oocyte secreted growth factors that are involved in ovarian follicle development. Studies on other mutations with large effects on ovulation rate have shown that increased ovulation is associated with earlier acquisition of gonadotrophin (LH, FSH) receptors on granulosa cells and associated changes leading to oestradiol-active follicles that are ovulated at smaller sizes. The objective was to characterise the expression of gonadotrophin receptor genes (FSH-R and LH-R) and steroid related genes (aromatase and steroidogenic acute regulatory protein (StAR)) in healthy follicles during pro- and early oestrus in relation to carrier status for mutations in BMP15 and GDF9 genes.

Adult Cambridge ewes heterozygous for *FecX<sup>G</sup>* (BMP15, n=7) or *FecG<sup>H</sup>* (GDF9, n=7), or wildtype (=non-carriers) (WT, n=7) were synchronised using a 12-day intra-vaginal progestagen pessary (Chronogest, 30mg Cronolone, Intervet, NL). Ewes were humanely euthanised at either 12 or 36 h post pessary removal. Ovaries were recovered within 5 min, weighed and all follicles ≥3 mm dissected. Each follicle was placed on ice until processed. Follicular fluid was aspirated and stored at -20 °C. Granulosa cells were then floated off the follicle in sterilized PBS, centrifuged at 4 °C for 1 min, snap frozen in liquid N and stored at -80 °C for RNA extraction. The theca layer was dissected from the follicle wall and snap frozen in liquid N and stored at -80°C for RNA extraction. Follicular fluids were assayed for estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>) to identify healthy follicles using validated radioimmunoassays. Total RNA was isolated using RNeasy Plus Mini extraction kits (Qiagen, UK) and cDNA obtained using Superscript™ III First-Strand synthesis system for real-time polymerase-chain reaction (RT-PCR; Invitrogen Life Technologies, CA). RT-PCR to quantify expression level of target genes was run in duplicate using appropriate primers. The resulting data were analysed using the 2<sup>-ΔC<sub>T</sub></sup> method. An endogenous control (18s rRNA PDAR (ABI 4310893E) labelled Vic on the 5' end) allowed for the calculation of ΔC<sub>T</sub> (target gene mean C<sub>T</sub> – endogenous control C<sub>T</sub>). Mixed model (fixed effects for time and genotype and random effect for animal) procedures were used for statistical analysis of expression data.

Mean ovary weights for WT, GDF9 and BMP15 ewes were 1.2, 0.9, and 1.3 (s.e. 0.14) g, respectively. The difference between WT and BMP15 was not significant but GDF9 was significantly lower than WT and BMP15 (P<0.05). The total number of follicles in carriers (GDF9 and BMP15) was significantly (P < 0.05) greater than in WT; 6.4 and 6.9 v. 5.0 (s.e. 0.58). Carriers (GDF9 and BMP15) had more E<sub>2</sub>-active follicles than WT ewes (4.9, 5.3 v 1.9; s.e. 0.51; P < 0.001); the corresponding mean diameters were 4.5, 5.0 v 5.6 mm (s.e. 0.34).

There was no evidence that granulosa cell expression of either FSH-R, aromatase or StAR genes was associated with genotype. The expression of LH-R in granulosa cells occurred earlier (12 h v 36 h) in carriers than in non-carriers (Figure 6; interaction between time and genotype P<0.09). The expression of StAR and LH-R in theca cells was not affected by

genotype. The development of LH receptors on granulosa cells is a key step for follicles to become dominant so that they can continue to grow, in the face of declining concentrations of FSH by increased E<sub>2</sub> secretory capacity and thus survive to ovulate.



**Figure 6. RT-PCR LH-R expression in granulosa cells for genotype-X-time.** <sup>ab</sup> means without common superscript differ ( $P = 0.09$ ).

The fact that the number of E<sub>2</sub>-active follicles did not differ between the carrier genotypes but that *FecX<sup>G</sup>* carriers had a significantly lower ovary weight and had the lowest diameter for E<sub>2</sub>-active follicles suggests that some E<sub>2</sub>-active follicles were not recovered from these ewes because they were <3 mm. This is supported by the fact that and *FecG<sup>H</sup>* carriers have a higher ovulation rate than *FecX<sup>G</sup>* carriers. Consequently it is proposed that *FecG<sup>H</sup>* carriers acquire LH receptors at a smaller size than *FecX<sup>G</sup>* carriers.

RMIS No. 4785

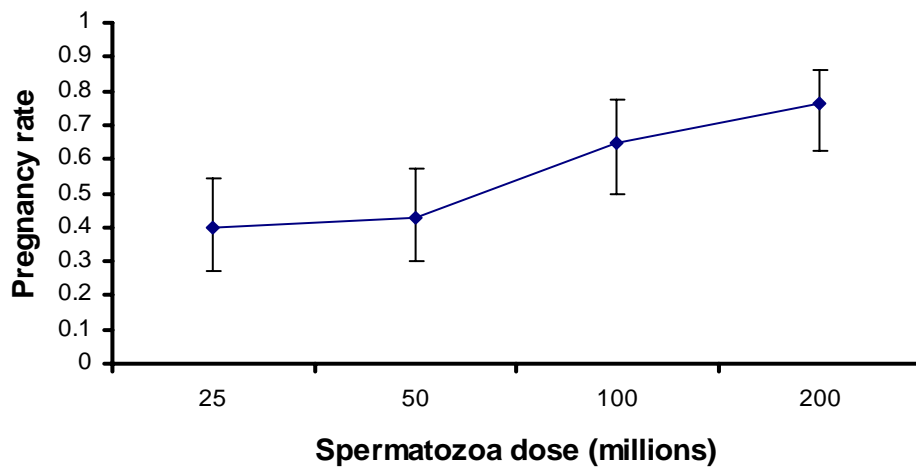
Haycock, H.<sup>1</sup>, Hanrahan, J.P., Sweeney, T.S.<sup>1</sup>, Roche, J.F.<sup>1</sup> and Crowe, M.A.<sup>1</sup>

<sup>1</sup>UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

### Effect of sperm dose on pregnancy rate in sheep

Rapid genetic progress in sheep is dependent on the availability of effective and efficient techniques for artificial insemination (AI). Currently, only a small number of inseminations per ejaculate (~10) are possible for use in cervical inseminations given that standard procedures usually employ between 200 and 400 x 10<sup>6</sup> sperm per inseminate. The ability to use low sperm doses would facilitate greater use of high genetic merit rams in breeding programmes. The objective of this study was to examine the effect of sperm dose on pregnancy rate in ewes cervically inseminated with fresh semen. Belclare (n= 52), Scottish Blackface (n=75), Scottish Blackface x Belclare (n=38) and Texel/Texel-cross ( n=26) ewes were synchronised with FGA pessaries for a 12-day period and received 500 i.u. eCG intramuscularly at pessary removal. Semen was collected from 4 rams on the morning of insemination and diluted with a skim-milk-egg yolk extender to give an insemination dose of i) 25 x 10<sup>6</sup>, ii) 50 x 10<sup>6</sup>, iii) 100 x 10<sup>6</sup> and iv) 200 x 10<sup>6</sup> spermatozoa and held at 15°C (approximately 6 h) until insemination at 52 h post pessary removal. Ewes were slaughtered 26 days later and ovulation rate and number of foetuses were recorded. The proportion of ewes pregnant is shown in Figure 7. The effect of sperm dose was highly significant ( $P < 0.001$ ). The differences among the dose levels was essentially linear as the quadratic term did not approach significance ( $P > 0.5$ ). There was also a significant effect of ewe breed on pregnancy rate ( $P, 0.05$ ); the estimated values were 0.57, 0.71, 0.57 and 0.40 for Belclare, Scottish Blackface, Belclare x Scottish Blackface and Texel/Texel cross ewes, respectively. There was no evidence for any breed by dose interaction. The results indicate that lowering the dose of spermatozoa adversely affects pregnancy outcome when ewes are cervically inseminated with fresh semen. This remains a limiting factor affecting the rapid

dissemination of identified superior genotype for this method of insemination. Previous studies have shown a highly significant effect of ewe breed on pregnancy rate when frozen-



**Figure 7. Effect of sperm dose on pregnancy rate (vertical bars represent the 95% confidence interval).**

thawed semen is used. However, the effect of ewe breed on pregnancy rate following insemination with fresh semen is somewhat surprising as this has not been noted in previous experiments.

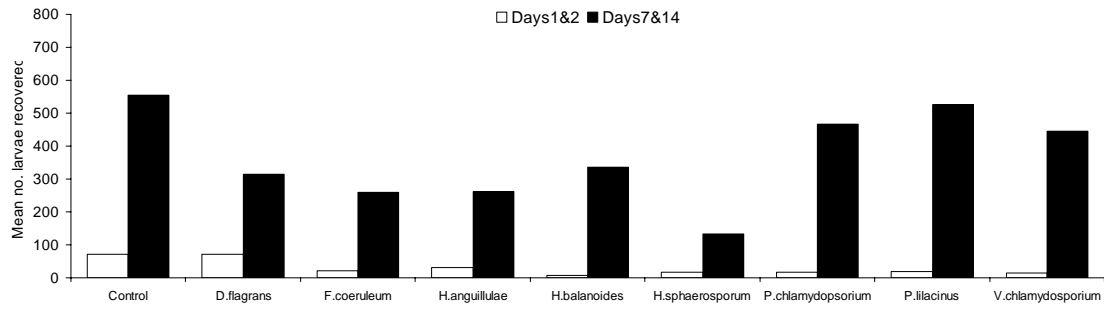
Donovan, A., Lally, T. and Hanrahan, J.P.

RMIS No. 5081

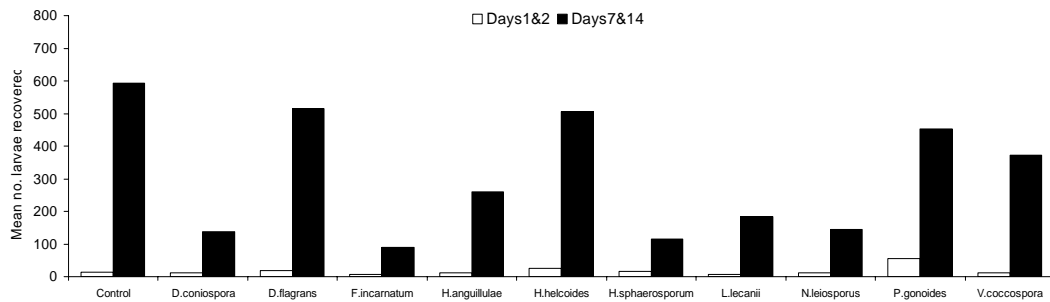
**Effect of nematophagous fungi on *Teladorsagia circumcincta* eggs in ovine faeces maintained at 16°C**

The use of biocontrol agents in parasite control is becoming an increasingly desirable option given the issue of anthelmintic resistance. Nematophagous fungi attack and kill nematodes using them as a source of nutrients and have potential as biocontrol agents. Previous work (Research report 2005 p135-137) identified 12 out of 16 isolates (native to Ireland /UK) with a clear ability to affect the development of *T. circumcincta* eggs incubated at 24°C. The objective of this present study was to evaluate the effect *in vitro* of the same 16 isolates of endoparasitic nematophagous fungi on *Teladorsagia circumcincta* eggs at 16°C. A further four isolates (not previously examined) were tested at 16°C and 24°C.

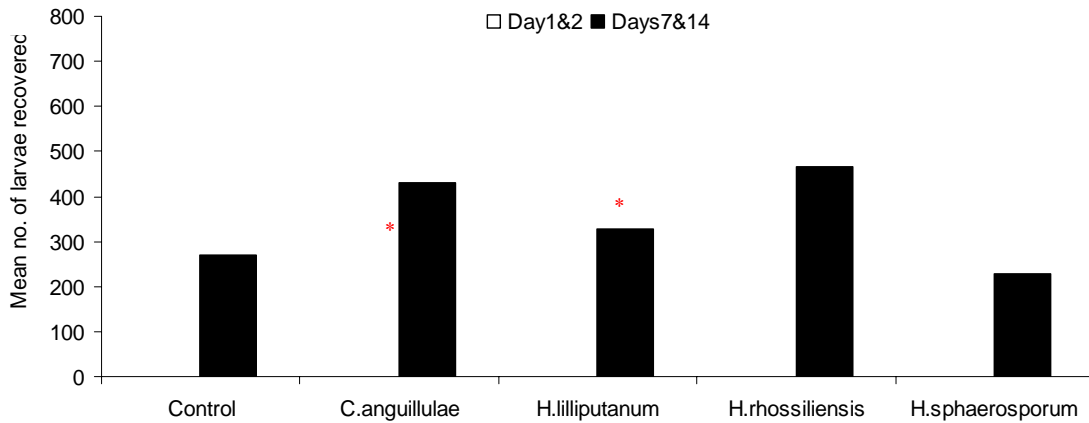
The methodology was the same as described previously (Research Report 2005 p135-137) with eight replicates for each species and negative controls (containing no fungi). *Duddingtonia flagrans* was included as a positive control. The coprocultures plus/minus fungal treatment were incubated for 1, 2, 7 and 14 days. Larvae were subsequently recovered (Baermann apparatus) and quantified. Due to space/time constraints the 20 fungal species were examined in 3 batches (Batch C, D and E). Batch C involved 8 species at 16°C (including *D. flagrans*). Batch D consisted of 10 species at 16°C (including *D. flagrans* and 2 species previously tested in Batch C). Batch E consisted of 4 species, not previously investigated, that were examined at both 16°C and 24°C for 1, 2, 7 and 14 days. The results are in Figures 8 to 11.



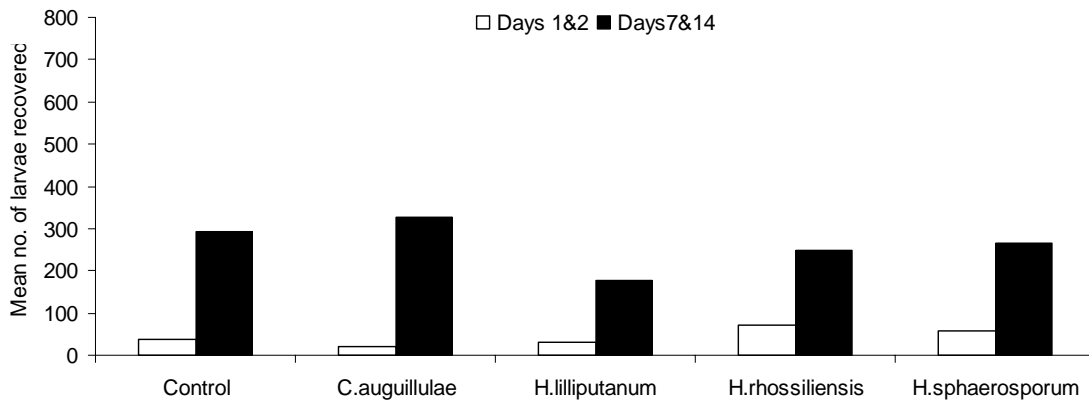
**Figure 8. Batch C: Effect of fungal species on larval recovery for days 1 and 2, 7 and 14 incubated at 16°C.**



**Figure 9. Batch D: Effect of fungal species on larval recovery for days 1 and 2, 7 and 14 incubated at 16°C.**



**Figure 10. Batch E: Effect of fungal species on larval recovery for days 1 and 2, 7 and 14 incubated at 16°C.**



**Figure 11. Batch E: Effect of fungal species on larval recovery for days 1 and 2, 7 and 14 incubated at 24 °C.**

**Table 21: Summary of the reduction in number of larvae in fungal treated faecal material compared to controls**

Fungal species	% reduction at 16 °C		% reduction at 24 °C <sup>1</sup>	
	Days 1&2	Days 7&14	Days 1&2	Days 7&14
<i>Drechmeria coniospora</i>	21	77	87	97
<i>Harposporium anguillulae</i> *	37	54	95	99
<i>Fusarium coeruleum</i> *	70	53	63	63
<i>Fusarium incarnatum</i> *	43	85	73	86
<i>Haptocillium sphaerosporum</i> *	38	78	96	99
<i>Nematoctonus leiosporus</i>	25	75	42	46
<i>Paecilomyces lilacinus</i> *	73	5	54	51
<i>Verticillium chlamydosporium</i> *	80	20	69	75
<i>Duddingtonia flagrans</i> *	6	28	53	64

<sup>1</sup> Results from experiment described in research report 2005.

\* Species with ability to produce chlamydospores and therefore chosen for animal trials, to assess survival through the GI tract.

The ability of these species to pass through the gastrointestinal tract of lambs will now be examined in six isolates, highlighted in Table 21, that meet the following criteria namely; (1) the ability to produce highly resistant spores (chlamydospores) and (2) showed a particular detrimental effect on the nematode development.

Kelly, P.<sup>1</sup>, Good, B., Hanrahan, J.P., De Waal, T.<sup>1</sup> and Mulcahy, G.<sup>1</sup>

RMIS No. 5387

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**Development of a PCR diagnostic test for the rapid detection of the nematophagous fungus *Duddingtonia flagrans***

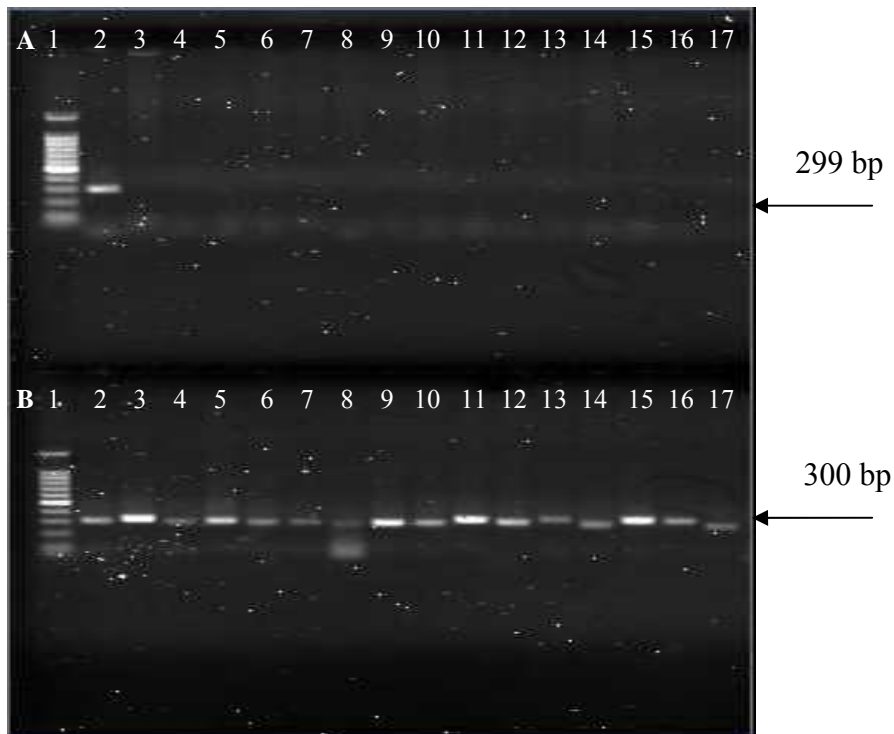
The nematophagous fungus, *Duddingtonia flagrans* is a potential biocontrol agent against nematode parasites of ruminants. Improved methods for the accurate and sensitive detection of *D. flagrans* would significantly aid in the evaluation of this fungus as a potential biocontrol agent in the field. Primers that target the internal transcribed spacer (ITS) regions combined with the polymerase chain reaction (PCR) technology can be used to develop rapid, accurate and sensitive species-specific diagnostic tests for a broad range of organisms. The

## Athenry Research Centre

development of a species specific PCR diagnostic test for the rapid detection of *D. flagrans* was undertaken.

Sixteen endoparasitic fungal species, (see Research Report 2005 p 135-137) were obtained from Centraalbureau voor Schimmelcultures (CBS), Netherlands. Genomic DNA was isolated by mechanical disruption and enzymatic digestion. Briefly, approximately 500 mg of liquid nitrogen frozen fungal mycelium was homogenised in 3 ml of PBS (pH 7.0) containing 3 mg of lysing enzyme (Sigma) and incubated at 37°C for 3 h. This was followed by a 1 h incubation at 65°C in extraction buffer (50 mM Tris HCl, 100 mM EDTA, 100 mM NaCl<sub>2</sub>, 1 % SDS, pH 8.0) containing 3 mg of proteinase K. DNA was extracted with phenol and chloroform and precipitated using 0.6 volumes of isopropanol. The quantity and quality of the DNA was determined by absorbance at 260 and 280 nm. Primer design was based on internal transcribed spacer (ITS) DNA sequences obtained from GenBank/EMBL databases. The ITS1 and ITS2 sequences of *D. flagrans* were aligned with ITS1 and ITS2 sequences derived from the closest related fungal species using ClustalW. From this alignment two *D. flagrans* species specific primers were designed, DFITS1 and DFITS2. The optimum PCR reaction consisted of 100 ng of genomic DNA, 1X PCR buffer (Promega WI USA), 0.2 µM dNTPs, 4 mM MgCl<sub>2</sub>, 100 ng of each primer and 2.5 U of *Taq* DNA polymerase (Promega WI USA) in a final reaction volume of 50 µl. Optimised PCR amplification conditions consisted of 34 cycles of 95 °C for 30 s, 66.6 °C for 30 s, and 72 °C for 30 s. PCR products were separated on 1.5 % EtBr stained gels and visualized using a Molecular Imager FX (Bio-Rad). The identity of the PCR product was confirmed by DNA sequencing. The primers used for universal fungal amplification were UR (5'-TCCTCCGCTTATTGATAGC-3') and UF (5'-GTGAATCATCGAATCTTTGAA-3'). DNA extraction from soil samples (5 g) was performed using the UltraClean™ Mega Soil Kit according to manufacturer's instructions (Mo Bio, Laboratories). Humic acid and other PCR inhibitors were removed using the PowerClean™ DNA Clean-up Kit (Mo Bio, Laboratories).

The sensitivity of the PCR assay was determined in two ways. In the first approach, a soil sample (5 g) was spiked with 10<sup>6</sup> *D. flagrans* chlamyospores and subjected to DNA extraction as described above. The extracted DNA was diluted to provide the equivalent of 120, 50, 10, 5 and 1 chlamyospore and then subjected to PCR analysis. In the second approach, DNA was extracted from individual soil samples (5 g) spiked with 5 x 10<sup>5</sup>, 5 x 10<sup>4</sup>, 5 x 10<sup>3</sup>, 5 x 10<sup>2</sup>, 50 and 5 chlamyospores. The extracted DNA was subjected to PCR and the presence or absence of the *D. flagrans* specific 299 bp PCR product determined by agarose gel electrophoresis.



**Figure 12.** Specificity of the PCR assay with genomic DNA isolated from 16 different fungal species. Panel ‘A’ represents PCR assay using *D. flagrans* species specific primers. Panel ‘B’ represents control PCR assay using fungal universal primers. Lane 1. 100 bp marker, 2. *D. flagrans*, 3. *Arthrotritys oligospora*, 4. *Harpsosporium lilliputanum*, 5. *Harpsosporium helicoides*, 6. *Haptocillium sphaerosporum*, 7. *Paecilomyces lilacinus*, 8. *Haptocillium balanoides*, 9. *Verticillium sphaerosporum*, 10. *Drechmeria coniospora*, 11. *Pochonia. chlamydosporium*, 12. *Fusarium coeruleum*, 13. *Verticillium coccospora*, 14. *Fusarium incarnatum*, 15. *Verticillium chlamydosporium*, 16. *Pyricularia higginsii*, 17. *Fusarium oxysporum*.

*D. flagrans* species specific primers were found to be highly specific when tested against 15 different fungal species (Figure 12, Panel A). A single PCR product of the expected size 299 bp was exclusively amplified from *D. flagrans* demonstrating the specificity of the PCR assay. The control PCR reaction using universal fungal primers was positive for all fungal isolates (Figure 12, Panel B). The PCR assay was also positive when tested on four different *D. flagrans* isolates (data not shown). Soil samples containing the equivalent of 10 chlamydo spores were sufficient for reliable amplification of the *D. flagrans* specific PCR product (Figure 13).



**Figure 13.** Sensitivity of the assay using DNA dilution equivalents. Lane 1. 100 bp ladder, Lanes 2, 3, 4, 5, 6 represent 1, 5, 10, 60 and 120 chlamydo spores respectively.



**Figure 14. Sensitivity of the assay using different chlamydo-spores concentrations per gram of soil. Lane 1. 100 bp ladder, Lane 2. Control – Water. Lanes 3, 4, 5, 6, 7 and 8 represent  $10^5$ ,  $10^4$ ,  $10^3$ ,  $10^2$ , 10 and 1 chlamydo-spores, respectively.**

However when the DNA extraction and PCR amplifications were performed on soil samples spiked with known quantities of chlamydo-spores the detection level was found to be 100 chlamydo-spores per gram of soil (Figure 14). The sensitivity achieved in this second method was lower but takes into account DNA losses that occur during the DNA extraction and clean up procedures and it is suggested that this is a more accurate measurement of assay sensitivity for field samples than a method which determines sensitivity based on serial dilution of DNA which overestimate the true sensitivity of PCR assays.

The PCR diagnostic test developed in this study provides a rapid ( $< 8$  h), accurate and sensitive test for *D. flagrans* which will enhance the evaluation of this fungus as a nematode biocontrol agent in the field. This test will now be used to determine whether *D. flagrans* is present in Ireland. Ten sheep farms (Kilkenny  $n=4$ , Wicklow  $n=6$ ) were visited in early September. Faecal, leaf litter and soil samples ( $n=5$ ) were collected at random from permanent sheep pasture (one field) on each farm.

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### **Anthelmintic efficacy on ovine parasitic nematode populations on the Teagasc sheep farms**

Results from a composite faecal egg count (determined by FECPAK method) revealed an efficacy problem in post administration with a benzimidazole product on the Athenry farm (Research Report 2005, pg 143-146). More detailed investigations were undertaken subsequently to establish the reason for this apparent lack of efficacy. Faecal samples were taken from lambs pre and post treatment and faecal egg counts determined for each lamb according to the modified McMaster procedure. FEC was distinguished as *Nematodirus* ( $FEC_{NEM}$ ) and other trichostrongyles excluding *Nematodirus* and *Strongyloides papillosus* ( $FEC_{OT}$ ). Criteria for inclusion in the study were as follows; the FEC taken prior to anthelmintic administration was  $>100$  epg and a post treatment FEC was available. All animals studied were lambs born in 2005 and flocks tested were those at Athenry (purebred Suffolk & Texels) and Leenane (purebred Scottish Blackface). Anthelmintics were administered in accordance with manufacturer's instructions. Results are shown in Table 22.

The results indicate benzimidazole resistance in the 'other trichostrongyle' nematode populations in both the Leenane and Athenry flocks. In Leenane, the level of resistance is greater. Where there was a *Nematodirus* FEC available for assessment i.e. Athenry flock there was no indication of a reduction in treatment efficacy against *Nematodirus*.

**Table 22: Results of the FECRT tests from the Athenry and Leenane flocks in 2005**

Flock	Variable	FEC <sup>1</sup>		% reduction (95% CI)	Active Ingredient
		Pre <sup>2</sup>	Post <sup>3</sup>		
Athenry (n = 20)	FEC <sub>OT</sub>	925	313	71.2 (63.4 – 89.2)	Mebendazole <sup>4</sup>
Athenry (n = 10)	FEC <sub>NEM</sub>	310	8	97.0 (92.0-101.3)	Mebendazole <sup>4</sup>
Leenane <sup>5</sup> (n = 9)	FEC <sub>OT</sub>	1550	2589	-103.5	Oxfendazole <sup>6</sup>

<sup>1</sup>FEC; faecal egg count. <sup>2</sup> pre anthelmintic administration. <sup>3</sup>post anthelmintic administration. <sup>4</sup>Chanazole, Chanelle Pharmaceuticals Manufacturing Ltd. <sup>5</sup>Nematodirus FEC was too low (<100 egg for all animals).

<sup>6</sup>Systemex 2.265, Schering Animal Health Ireland.

Good, B., Hanrahan, J.P., Walsh, H. and O'Malley, L.

RMIS No. 5253

**Efficacy of levamisole on ovine parasitic nematodes and whether efficacy of benzimidazole is improved by fasting lambs prior to benzimidazole administration**

The aim of the present study was two-fold: to determine whether the efficacy of benzimidazole would be improved if animals were fasted prior to administration of benzimidazole and to establish the efficacy of levamisole. Belclare lambs (n = 50), 18 weeks of age approximately, that were co-grazed and had not received anthelmintic for a least 8 weeks prior to the trial were assigned at random to 3 treatment groups (Table 23).

**Table 23: Details of treatment groups**

Group	Treatment
A (n = 17)	Levamisole <sup>1</sup>
B (n = 17)	Benzimidazole <sup>2</sup>
C (n = 16)	Fasted for 24 h prior to benzimidazole administration

<sup>1</sup>Nilzan Drench Plus, Schering Plough Animal Health. <sup>2</sup>Systemex 2.265, Schering Plough Animal Health, Ireland.

Anthelmintics were administered with a syringe and the volume was based on lamb's individual body weight and manufacturer's instructions. Faecal samples were collected *per rectum* from each animal pre (day 0) and post (day 10) anthelmintic administration and faecal egg counts (FEC) determined using the modified McMaster method. FEC was distinguished as *Nematodirus* (FEC<sub>NEM</sub>) and 'other trichostrongyles' which excluded *Nematodirus* and *Strongyloides papillosus* (FEC<sub>OT</sub>). The criteria for inclusion in subsequent analysis were as follows: the FEC taken prior to anthelmintic administration was >100 eggs per gram and a post-treatment FEC was available. Faecal cultures (incubated at 24 °C for 9 days) were prepared for each treatment group. The FEC results are summarised in Table 24.

**Table 24: Results of the FECRT according to treatment groups**

Group	n	Species	FEC		% Reduction (95% CI)
			Pre <sup>1</sup>	Post <sup>2</sup>	
A	12	FEC <sub>OT</sub>	688	66	86.6 <sup>a</sup> (72.3-99.4)
	4	FEC <sub>NEM</sub>	188	0	100 (-)
B	14	FEC <sub>OT</sub>	604	224	53.3 <sup>b</sup> (66.3-82.0) <sup>3</sup>
	5	FEC <sub>NEM</sub>	240	0	100 (-)
C	11	FEC <sub>OT</sub>	555	266	46.6 <sup>b</sup> (53.2-81.9) <sup>4</sup>
	6	FEC <sub>NEM</sub>	183	0	100 (-)

<sup>1</sup>Prior to anthelmintic administration. <sup>2</sup>post anthelmintic administration. <sup>3</sup>Confidence interval does not include animals where there was an increase in FEC n=2. <sup>4</sup>CI does not include animals where there was an increase in FEC observed, n=3. <sup>ab</sup> means with different superscript letters are significant different (P<0.05).

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The results confirm the observation of benzimidazole resistance in ‘other trichostrongyle’ nematode populations at Athenry. The efficacy of benzimidazole was not improved by fasting the lambs prior to administration. The results also demonstrate a reduced efficacy of levamisole against ‘other trichostrongyle’ nematode populations. While the numbers of lambs with suitable *Nematodirus* egg counts are low, the results do suggest that both products are highly efficacious against *Nematodirus*.

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### The evidence for anthelmintic resistance in farms in Co. Kilkenny

There are only three broad-spectrum anthelmintics available to control parasite nematodes in grazing animals. Previous work on anthelmintic resistance in Irish lowland flocks has highlighted evidence for a considerable problem facing producers with respect to 2 of these broad-spectrum anthelmintic groups, namely, benzimidazole and levamisole (see Research Report 2005, pp 137-139). The present study was carried out to using a different method of anthelmintic resistance detection, namely the faecal egg count reduction test (FECRT). The producers whose flocks (n=7) were used belong to the “Kilkenny Sheep Discussion Group” and were involved in a Teagasc TET project.

On the initial visit, 30 ewe lamb replacements were chosen at random and individual management numbers and weights recorded. Individual faecal samples were taken, placed in air-tight bags, kept cool and posted back to the laboratory with 24 h of collection. Immediately post-sampling, 15 lambs were administered a benzimidazole product (Systamex 2.265, Schering Plough Animal Health, Ireland) and 15 lambs were given a levamisole product (Nilzan, Drench Plus, Schering Plough Ltd) according to manufacturers instructions. Between 10 and 14 days post-treatment a minimum of 10 lambs per treatment were resampled. Faecal egg counts (FEC) were performed according to modified McMaster technique and arithmetic means for each group of lambs calculated pre and post treatment. The criteria used to evaluate anthelmintic resistance were based on the World Association for the Advancement Veterinary Parasitology guidelines for detecting resistance (WAAVP, 1992). Anthelmintic resistance was considered present where the FEC reduction post-treatment was less than 95% and the lower limit of the confidence interval for the percentage reduction was less than 90%. Anthelmintic resistance is suspected if only one of these two criteria is fulfilled.

**Table 25: Lamb FEC (excluding *Nematodirus* and *Strongyloides papillosus*) pre and post treatment**

Flock ID	Benzimidazole		% reduction	Levamisole		% reduction
	Pre FEC <sup>1</sup>	Post FEC <sup>2</sup>		Pre FEC <sub>1</sub>	Post FEC <sub>2</sub>	
KK01	336	185	23.7*	585	6	99.1
KK02	377	232	24.7*	365	5.0	98.1
KK03	1254	719	42.2*	1092	85	89.0*
KK04	1800	1204	32.7*	1790	191	81.0*
KK05	745	205	56.8*	933	29.5	97.1
KK06	523	313	32.6*	536	50	83.7*
KK07	1015	225	73.6*	1154	24	96.3 <sup>†</sup>

<sup>1</sup>pre treatment FEC, eggs per gram, <sup>2</sup> post treatment FEC, eggs per gram, <sup>†</sup>Resistance suspected, \* Resistance.

The results (Table 25) show evidence for significant resistance to benzimidazole and levamisole in the worm population on 7 and 3 farms, respectively. The present finding corroborate with the results from earlier studies on lowland flocks (Research Report 2002, 2005) and confirms the unsustainable nature of a chemotherapeutic dominated approach to nematode control.

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*RMIS No. 5253*

### **The effectiveness of copper oxide wire particles as an anthelmintic in purebred Suffolk lambs exposed to a natural nematode challenge post weaning**

Resistance to nematode parasites of sheep to broad spectrum anthelmintics is one of the main drivers in research on alternative solutions for parasite control. Previous work at this centre has shown clear differences between the Suffolk and Texel breeds in susceptibility to gastrointestinal nematode infection (Research report 1999, pp 24-25, 2000, pp 21-23). The greater susceptibility of the Suffolks has implications in the Irish industry where it plays a dominant role as both as a terminal sire and in the genetic makeup of the ewe population. Previous work has shown some beneficial anthelmintic effects following the administration of copper oxide wire particles. The aim of this study was to evaluate the potential of copper oxide wire particles (COWP) as an anthelmintic for lambs that were exposed to a natural nematode challenge post weaning.

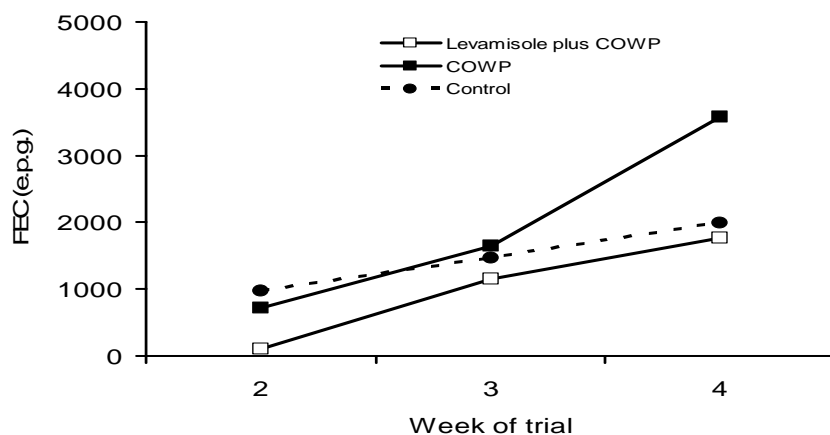
Purebred Suffolk lambs (n=24) were randomly assigned to one of 3 treatment groups post weaning; A: administered levamisole (5 ml/10 kg; Nilzan Drench Plus, Schering Plough Animal Health) plus COWP (2.0 g/lamb; Copasure, Animax Ltd.), B: COWP and C: control (no COWP or levamisole administered) and c grazed. Strongyle faecal egg counts were determined for each lamb on a weekly basis. Faecal egg counts were distinguished as *Nematodirus* (FEC<sub>NEM</sub>), *Strongyloides papillosus* (FEC<sub>Spap</sub>) and 'other trichostrongyles' (FEC<sub>OT</sub>). Coprocultures for each treatment group were also completed to determine information on species composition of the FEC. Animals were weighed pre and post trial. FEC (weeks 2, 3 & 4) was analysed using Proc Mixed of SAS (SAS, 1989) with animal within treatment as a random effect and initial FEC (taken pre-treatment) as a covariate. The model had fixed effects for treatment, week and treatment-by-week interaction. Prior to analysis, the FEC data were transformed to logarithms ( $\ln(x+1)$ ) to normalise the data.

On the basis of rising FEC values and growing clinical evidence of infection it was decided to shorten the trial by 2 weeks and all animals were treated with levamisole at week 4. Least squares means (weeks 2, 3 & 4) for FEC<sub>NEM</sub>, FEC<sub>Spap</sub> and FEC<sub>OT</sub> according to treatment group are shown in Table 26. There was a significant treatment effect for FEC<sub>Spap</sub> (P<0.05) and FEC<sub>OT</sub> (P<0.01). There was a significant week and treatment-by-week interaction for FEC<sub>OT</sub> (P<0.005). The treatment by week means for FEC<sub>OT</sub> is shown in Figure 15. As expected FEC<sub>OT</sub> value for the Levamisole treated group is negligible 2 weeks after treatment. Subsequently an increase in FEC<sub>OT</sub> is observed in response to nematode challenge and the infection becoming patent. Larvae recovered from the coprocultures were predominantly species that reside in the small intestine (Figure 16). There was no treatment effect on final weight. The mean weight ( $\pm$  s.e.) for animals in treatment groups A, B and C were 51.2  $\pm$  0.78, 50.5  $\pm$  0.82, 48.6  $\pm$  0.92 kg respectively.

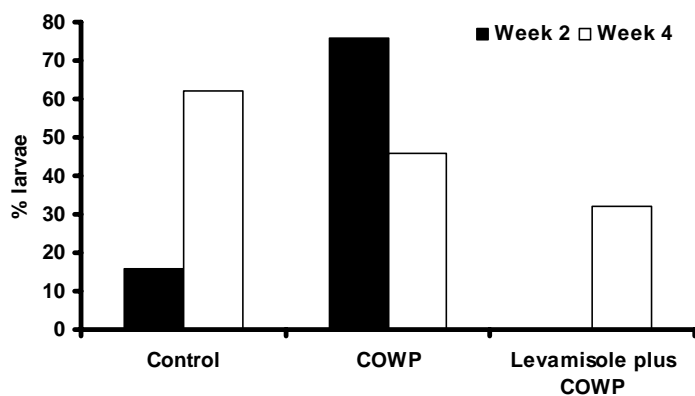
**Table 26: Least squares means and standard errors (log scale) (weeks 2,3 & 4 ) of faecal egg counts by treatment**

	Treatments		
	Levamisole + COWP	COWP	CONTROL
FEC <sub>NEM</sub>	2.4 <sup>a</sup> (0.69)	3.4 <sup>a</sup> (0.68)	3.1 <sup>a</sup> (0.76)
FEC <sub>Spap</sub>	3.7 <sup>a</sup> (0.26)	4.9 <sup>b</sup> (0.26)	4.3 <sup>ab</sup> (0.28)
FEC <sub>OT</sub>	6.4 <sup>a</sup> (0.20)	7.4 <sup>b</sup> (0.20)	7.3 <sup>b</sup> (0.22)

<sup>ab</sup> Means with different superscript letters, within rows are significantly different (P< 0.05)



**Figure 15. Least squares means (back transformed) for 'other trichostrongyle' FEC for each treatment group by week.**



**Figure 16. Percentage of larvae identified from faecal culture as abomasum in origin.**

The results of the present study indicate that administration of copper oxide wire particles in lambs post-weaning had no effect on faecal egg count. However, the main species identified from faecal cultures were those located in the small intestine which, by their location, would not be susceptible to the effects of COWP.

Good, B., Hanrahan, J.P., Glynn, A. and Walsh, H.

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**The epidemiology of *Lymnaea truncatula* and the prevalence of *Fasciola hepatica* within hosts (snail and sheep) on the Teagasc hill sheep farm: preliminary results**

Fascioliasis (liver fluke disease) is caused by large numbers of immature stages of the parasite *Fasciola hepatica* through the liver and or the presence of adult flukes (blood feeders) in the bile ducts of liver and therefore can impose serious economic penalties. Because, the life cycle is complex requiring an intermediate host the mud snail, *Lymnaea truncatula* and

several free-living stages, the incidence of fascioliasis is influenced by climate especially rainfall and temperature which affects the development of *F. hepatica* and the availability of infected snails. As a result, the disease is more prevalent in the wetter areas of Ireland i.e. counties along the western sea board. Traditionally, liver fluke was known to have a distinct seasonal pattern with the challenge on pasture (as a result of abundant infected snails to complete the life-cycle) being greatest in late summer/autumn (following a wet summer) which would result in outbreaks of clinical fascioliasis in early/mid winter. In recent years however, there has been anecdotal evidence of an increase in the incidence of the disease mid-summer and in areas that were previously considered less suitable for the preservation of snail habitats. The reason for the change in the epidemiology of liver fluke may perhaps reflect changing weather patterns in particular milder wetter winters which would be favourable to the survival of the intermediate host the mudsnail and the free-living stages of the fluke. The objective of this study is to collect up to date baseline data on the population dynamics of *L. truncatula*, its survival and infection with *F. hepatica* and subsequent infection patterns in the final host (sheep).

Snail populations, their abundance size and the prevalence of *F. hepatica* infection in both snails and sheep were monitored on the Teagasc hill sheep farm near Leenane Co. Mayo. Following a survey of potential snail habitats on the farm, 4 sites were identified on the lowland pasture and revisited every 2 weeks to monitor the snail population i.e. their abundance, size and infection level. *F. hepatica* infection in snails was determined via dissection and PCR based procedures. Prevalence within ewe and lambs grazing the greenland or upland areas was monitored from a cohort (randomly selected at the beginning of the sampling period) on a monthly basis. Faecal and blood samples were taken to determine fluke egg count (per gram of faeces) and antibodies to fluke infection, respectively.

Snail abundance and *F. hepatica* infection within snails on the four sites is shown on Figure 17. Snail populations peaked later than expected and this may be explained by the drier conditions experienced during the summer which would be unfavourable to snail proliferation. Peak infection in snails was observed in November. Infection became apparent in lambs earlier in the lowland flock (July) compared to the hill flock (September). FEC was negligible in most cases (0 to 19 epg) with prevalence increasing as the season progressed. Prevalence was highest (53%) in lambs exposed to greenland only. Prevalence (positive FEC) in ewes (hill and low flock) ranged from 30 to over 90 %. Prevalence was highest in both flocks by December.

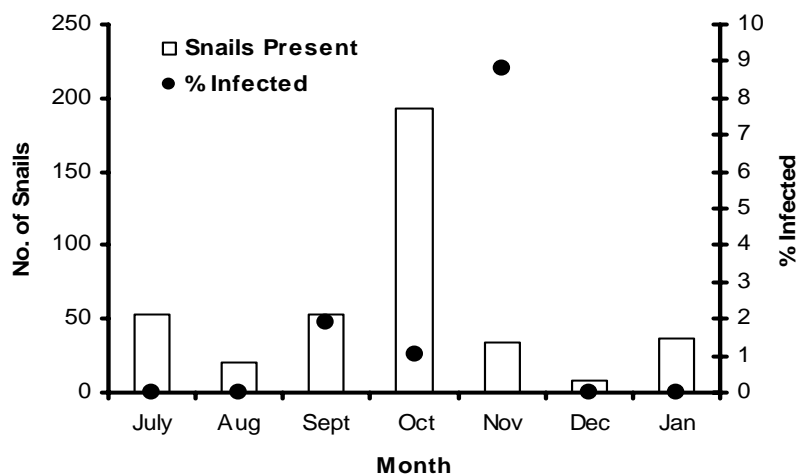


Figure 17. Snail abundance and numbers infected with *F. hepatica* at the four study sites.

## Athenry Research Centre

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