Effect of biotin supplementation on white line lesions and animal performance in sheep

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Executive summary

Lameness is a common problem in UK sheep flocks and causes significant loss of production and poor welfare. In the majority of flocks, foot rot and scald are the most common causes of lameness accounting for approximately 90% of foot lameness in the national flock. However white line disease (or shelly hoof) is common in many flocks (Phythian et al. 2011), and poor hoof integrity may be a precursor to other infections including foot rot.

It has been suggested that the B vitamin biotin may have a role to play in prevention of white line disease in sheep as it does in other species. Biotin is an essential nutrient for ruminants and rumen bacteria. It is required for rumen fermentation of dietary carbohydrate to propionic acid and for the conversion of propionic acid to glucose by the liver. Hoof horn formation requires biotin for the production of keratin and for the production of intracellular cement that bonds together hoof horn cells to form a semi-waterproof barrier to the environment.

Published research indicates that biotin supplementation can be beneficial as part of a control programme to reduce lameness in cattle and it has also been reported to increase milk production of dairy cows. A small number of studies have investigated milk and hoof health responses to supplementary biotin in sheep (Bampidis et al., 2007, Christodoulou et al., 2006) and the effect of biotin supplementation to ewes on lamb growth rate (Margerison, 2005). However further work is needed to assess the impact of biotin over the longer term on lameness and hoof health in sheep under UK conditions.

The objectives of the study reported here were to evaluate the effect of oral biotin supplementation on white line (WL) lesions and lamb performance in a lowland sheep flock.

A randomised split flock trial was carried out in a commercial North Country Mule flock with a history of white line disease in replacement ewe lambs. During the year of study the feet of 302 animals were repeatedly scored using a categorical scoring scale. Animals with WL lesions in one or more feet (n=260) and those without lesions (n=42) were randomly allocated to one of three treatment groups in January 2012;

- Control – no supplementation,
- Zinc – zinc-based rumen bolus and
- Biotin - a biotin and zinc bolus

Sheep were managed as a single group throughout the study. At four-monthly intervals, sheep were weighed, re-bolused and individual feet scored. They were subsequently mated to lamb in March 2013. Lamb performance data from birth (March 2013) to weaning (July 2013) was assessed for ewes scanned as carrying twins.

The majority of sheep were observed with WL lesions at the start (86%) and throughout the trial period although few were recorded with severe lesion scores at any assessment visit. There was some evidence that the proportion of sheep observed with white line lesions in January 2012 differed depending on their farm of origin. Regression analysis identified no significant difference ($p>0.05$) between the proportion or severity of WL scores recorded in Control, Zinc or Biotin groups at any of the four assessment visits. In feet observed with lesions in January 2012 (n=763)

there was some reduction in the percentage of WL lesions across all three treatment groups, although significant treatment differences were not observed.

Similarly an analysis of feet with no lesions present (score 0) in January 2012 (n=445) looked at the proportion that subsequently developed lesions (Score 1-5). Treatment differences were not observed at any sampling date with 25 – 30% being observed with lesions by the end of the study. An analysis of the foot data for 42 sheep that were lesion free at the start highlighted that by January 2013 just under half (48%) had retained their lesion–free status throughout the whole study period and these were evenly distributed across the treatment groups.

Analysis of live weight and live-weight gain data for study ewes confirmed that there were no treatment effects on ewe performance at any point during the study.
Twin-bearing ewes lambed indoors from 27th February 2013 and at birth lambs averaged 4.4 kg with weights being similar for all treatments. All lambs were creep fed. Lambs were weighed in May at 10 weeks of age and prior to weaning in July 2013. Lambs from ewes supplemented with biotin were significantly heavier at 10 weeks of age (23.7 kg) than Zinc (22.3 kg) or Control (21.8 kg) lambs (p=0.002) and had significantly higher growth rates (269, 251 and 245 g/d respectively) (p=0.003) from birth to 10 weeks.

The value of the additional live weight seen in Biotin lambs compared to Control lambs was estimated to be worth £4.60 (assuming £2.42/kg lwt) at 10 weeks of age. By weaning however the advantage to the Biotin treatment had disappeared with DLWG from 10 weeks to weaning (252 g/d) and weaning weight (36.1 kg) being similar for all treatments. Greater dependence on creep feed rather than milk may explain this levelling off in performance across treatments by weaning but the results do indicate superior performance in lambs born to ewes that received biotin which is likely to have been due to higher milk yield in the first 10 weeks of lactation.

Conclusions

This study identified that compared to control animals, four-monthly oral supplementation with a bolus releasing 82 mg/day available zinc only or 5mg/day biotin and 82 mg/day zinc did not have a significant effect on the severity of WL scores.

Biotin did not appear to facilitate healing of white line lesions or protect sheep against white line disease in this study.

As the study was conducted in a single lowland flock of replacement ewe lambs with relatively mild lesions it may be useful to evaluate the effect of biotin in a larger population with more severe lesions over a longer study period.

The effect of environmental/climatic conditions and the role of genetics in white line disease of sheep also warrant further research. The weather was particularly wet in 2012 -13 and this may have influenced the effect of biotin on WL lesions. Significant differences in the level of lesions were seen between sheep sourced from different farms in this study indicating that environmental or genetic factors may play an important part.

The cost of a biotin bolus was estimated at £1.60/head per dosing occasion equating to a cost of around £6.40 for this trial. However if the study had solely looked at lamb growth rate a single bolus administered in January would have been sufficient.

Supplementing ewes with biotin had a significant positive effect on lamb performance between birth and 10 weeks of age. Overall live weight at 10 weeks and DLWG to 10 weeks were lifted by 8% compared to the Control and Zinc treatments. Birth weight was unaffected by treatment. The value of the additional live weight of Biotin lambs at this point was estimated at £4.60/hd assuming £2.42/kg live weight.

Performance to weaning was similar for all treatments but is likely to have been strongly influenced by creep feeding of lambs. It may be beneficial to evaluate the effect of biotin on lamb performance in a flock where lambs do not receive creep feed.

Introduction

Lameness is a common problem in the UK sheep flock and causes significant loss of production and poor welfare. The Farm Animal Welfare Council Opinion on Lameness in Sheep (FAWC March 2011), states that further research into lameness in sheep is required and that an overall aim of reducing the national prevalence of lameness from around 10% to 5 % should be achieved within 5 years. A longer term aim of reducing prevalence to 2% or less should be achievable within 10 years.

In 80% of flocks, foot rot and scald (interdigital dermatitis with or without under-running separation of hoof horn from the underlying sensitive tissue) are the most common causes of lameness accounting for approximately 90% of foot lameness in the national flock. However white line disease (or shelly hoof) is common in many flocks (Phythian et al. 2011), and poor hoof integrity may be a precursor to other infections including foot rot.

The white line is a narrow zone at the junction of the wall horn and the sole horn of the hoof. Separation of varying extent is an extremely common problem of sheep's feet and is often confused with foot rot' (Winter and Phythian, 2011). It has been suggested that the B vitamin biotin may have a role to play in prevention of white line (and possibly other foot conditions) in sheep as it does in other species.

Biotin, a water-soluble, B-vitamin, is an essential nutrient for ruminants and rumen bacteria. Symptoms of biotin deficiency include lesions and malformations of keratin-containing structures such as foot pads in poultry, hooves, hair coat and fingernails (McDowell, 2000). Biotin is required for the rumen fermentation of dietary carbohydrate to propionic acid and for the conversion of propionic acid to glucose by the liver. Hoof horn formation requires biotin for the production of structural proteins (keratin) and for the production of intracellular cement that bonds together hoof horn cells to form a semi-waterproof barrier to the environment (Mülling et al., 1999). Both these factors affect the tensile strength and integrity of hoof horn, and ultimately the hoof health of cattle and sheep.

The role of biotin in preventing lameness in cattle

There are now many research papers published that indicate that biotin supplementation can be beneficial as part of a control programme to reduce lameness in cattle. The wealth of papers on this one vitamin probably exceeds publications on any other one area of lameness reduction. They include reports of the impact of biotin in reduction of all lameness (Fitzgerald et al., 2000), horn disorders including sole ulcer (Hagemeister and Steinberg, 1996), white line disease and fissures (Campbell et al., 2000) and digital dermatitis (Hagemeister and Steinberg, 1996; Distl and Schmid,1994).

Effects of biotin on milk production

Biotin is involved in many metabolic pathways directly involved with milk synthesis. Lactating cows must synthesise large amounts of glucose from propionate and if the activity of the enzyme propionyl-CoA carboxylase is limited by biotin availability (Zimmerly at al, 2001), a response to supplementary biotin is possible. High producing dairy cows would seem particularly susceptible to marginal B-vitamin status during lactation (Schwab et al., 2006).

Dietary supplementation of biotin has been reported to increase milk production of dairy cows (Bergsten et al., 2003; Bonomi et al., 1996; Enjalbert et al., 2008, Ferreira et al., 2007) Majee et al., 2003; Margerison et al. 2003; Midla et al 1998; Zimmerly and Weiss, 2001). Bonomi et al., (1996) supplemented cows with 0, 2, 6 or 10 mg biotin either in standard feed-grade or rumen-protected (lipid coated) form for the first five months of lactation. Biotin supplementation at 10 mg per day increased milk production by 1.5 kg per cow. Midla et al., (1998) and Bergsten et al., (2003) reported increased mature equivalent milk production (1.0 to 1.6 kg/d) respectively when cows were fed 20 mg of supplementary biotin. These two field studies were designed to study the effects of biotin on hoof health and were not specifically designed to evaluate milk yield responses.

Biotin studies with sheep

A small number of studies have investigated milk and hoof health responses to supplementary biotin in sheep (Bampidis et al., 2007, Christodoulou et al., 2006) and the effect of biotin supplementation to ewes on lamb growth rate (Margerison, 2005).

In a UK study, (Margerison, 2005) 120 twin bearing ewes were allocated to two treatments, supplementary biotin or not with two replicates per treatment. Concentrates supplemented with 7.5 mg/kg biotin were fed from 6 weeks prepartum at 200 g/h/d increasing at weekly intervals to a maximum of 850 g/h/d at lambing and until feed was withdrawn from the ewes at about 4 weeks postpartum. There were no significant differences in body condition at lambing, weaning or in change in body condition between ewes offered diets with no added biotin and with added biotin. At weaning, lambs weaned from ewes offered supplementary compound feed with added biotin were significantly heavier compared to lambs from ewes offered supplementary feed with no added biotin (31.0 kg v 32.2 kg; $P<0.05$).

ADAS, together with DSM (manufacturers of biotin) and Rumenco ran a small trial on feeding extra biotin to ewes pre-lambing (2011). 200 twin bearing ewes were fed 5 mg biotin/head per day (in a TMR ration) for 6 weeks pre-lambing and 2 weeks post lambing to assess the effect on lamb growth rate and directly compared to 200 ewes that did not receive biotin. Feeding biotin increased lamb birth weight by 8% ($p<0.001$) and improved lamb growth rate to 10 weeks of age by 6% ($p<0.001$).

In a study with lactating ewes, Christodoulou et al., (2006) supplemented either 0, 3, or 5 mg biotin in two experiments. In experiment 1, 72 ewes 7-26 weeks postpartum, biotin supplementation increased milk yield linearly (1064 g/d v 1171 g/d v 1443 g/d $P<0.001$) and milk fat concentration (54.2 g/kg v 58.3 g/kg v 62.3 g/kg $P<0.001$). In experiment 2, 72 ewes 24-36 wks postpartum responded similarly with increased milk yield (646 g/d v 684 g/d v 797 g/d $P<0.001$) and milk fat concentration (65.6 g/kg v 67.1 g/kg v 70.5 g/kg).

Bamphis et al., (2007) conducted a 12-month study to evaluate effects of biotin supplementation on lameness in sheep. Thirty non-lactating Chios ewes with existing lameness and physical appearance of hoof lesions (i.e., sole ulcer, sole haemorrhage, and heel erosion) were assigned to one of three treatment groups and fed 0.21 mg, 3.26 mg and 5.25 mg/day of biotin, respectively, with the diet. The hooves of all ewes were visually assessed every 4th week and hoof lesion score and locomotion score were recorded. Results demonstrate that hoof disorders in sheep are responsive to biotin supplementation, and that continuous biotin supplementation at 3.26 mg/day of biotin caused some healing of hoof lesions after 7 months but, at the end of the experiment, lesions were still present. However at 5.25 mg/day, hoof lesions healed within 7 months (with the healing being evident within 4 months) and improved locomotion.

Proposal

Limited evidence suggests that sheep respond to biotin supplementation. Milk yield and milk fat concentration have been increased significantly and/or lamb growth rates increased.

Further work needs to be done to assess the impact of biotin over the longer term on lameness and hoof health in sheep under UK conditions.

Objective

Primary objective:
To evaluate the effect of oral biotin supplementation on white line lesions in a lowland sheep flock.

Secondary objective:
To investigate the effect of supplementing ewes with oral biotin on the performance of twin lambs.

Materials and methods

Site and flock management

The study was carried out in a large commercial flock of North Country Mule ewes in the East Midlands. The flock had previously been observed to have a high incidence of white line disease in purchased ewe lamb replacements. The farmer's replacement policy is to source ewe lambs directly from three farms in the North of England each year and 'run these round' before putting them to the ram as yearlings. Ewe lambs are vaccinated with Footvax on arrival with subsequent annual boosters and hence foot rot was unlikely to have been a significant complicating factor in the flock.

In total 302 ewe lambs were allocated to the study in January 2012 and these were managed as a single group (picture above shows the study group in September 2012). They were subsequently mated to lamb in March 2013. Lamb performance data from birth (March 2013) to weaning (July 2013) was assessed for ewes scanned as carrying twins.

Experimental design

A single randomised split flock design with approximately 100 sheep per treatment. In total 302 North Country Mule ewe lambs were allocated to a one-year longitudinal study in January 2012.

Treatments

A zinc-based biotin bolus (developed by Agrimin Limited) was used in this study to ensure a regular supply of biotin, eliminating the need to feed biotin on a daily basis. To ensure that any treatment effects could be related to the biotin supplementation (rather than the zinc) a positive control consisting of a zinc-only bolus was included. The three treatments were:

1. Control – no supplementation,
2. Zinc – zinc-based rumen bolus (releasing 82 mg bioavailable zinc/head/day*)
3. Biotin - a biotin and zinc bolus (releasing 5 mg biotin and 82 mg zinc/head/day over four months).

* The contribution of zinc to the diet from the treatment boluses was approximately 82 mg/d assuming a relative bioavailability of zinc metal powder of 28% compared to zinc oxide and an active life for the bolus of up to 180 days.

Assessments

The feet of all sheep were scored by an experienced assessor using a categorical scoring scale (Table 1) at the start of the study (January 2012). Animals with white line lesions (score 1-5) in one or more feet (n=260) and those without (all feet score 0) (n=42) were randomly allocated to one of the three treatment groups. Treatment boluses were administered by Agrimin personnel (blind application so that farmer/assessors were not aware which sheep had biotin) and ewes colour marked by treatment group. At four monthly intervals sheep were re-bolused and individual feet scored by the same assessor who remained blind to the treatments (May 2012, September 2012 and final assessment in January 2013).
On each occasion a record was made of any other foot lesions seen (by type) e.g. interdigital dermatitis, foot rot etc. and any treatments given. The farmer also recorded any animals treated for lameness at other times plus records of deaths or culls.

Table 1. White line lesion scoring scale* (score 1 lesion shown)

Score	Description
0	No lesion observed/lesion healed
1	Minor separation of the white line (shelly hoof)
2	Moderate separation of the white line
3	Major separation of the white line
4	Discrete lesions in the white line with no separation
5	Active infection of the white line

* A full version of the white line lesion scoring scale can be found in Appendix 1.

All ewe lambs had electronic ear tags and, to aid data collection, foot scores and live weight data were entered onto a handheld EID reader with tailored data collection software (Agrident APR500, Border Software Ltd). Where sheep had lost the electronic identifier the ear tag number was entered manually.

The ewe lambs/ewes were weighed at each assessment date and were pregnancy scanned in January 2013. Lambs from twin bearing ewes were EID tagged and weighed at birth, in May (approx. 10 weeks) and at weaning. Additional records on sale dates, live weight at sale, carcass weights and carcass classification were collected but are beyond the scope of this report.

Blood samples were taken from a selection of ewes at the start of the study (January 2012), in May 2012 and January 2013. These were analysed for cobalt (Vit B12), copper, GSH-Px (indicator of selenium status) and zinc.

Statistical analysis

Foot lesion data were analysed using logistic regression to examine the proportion of feet with lesions present at each assessment date. In addition the chi square test was used to analyse lesion score distributions on individual dates and the pregnancy scanning data. Animal performance data were analysed using ANOVA (Genstat 8^{th} edition).

Results and discussion

The majority of sheep were observed to have white line lesions in at least one foot at the start of the study (86%) and throughout the trial period (May 75%, Sep 69% and Jan 2013 76%) although few feet were recorded with severe lesion scores at any assessment visit (Figure 1). The proportion of feet with white line lesions (score 1-5) was observed to fall up to Sep 2012 but by Jan 2013 the trend had reversed with more lesions seen (similar to May levels) largely due to an increase in Score 2 (moderate) lesions.

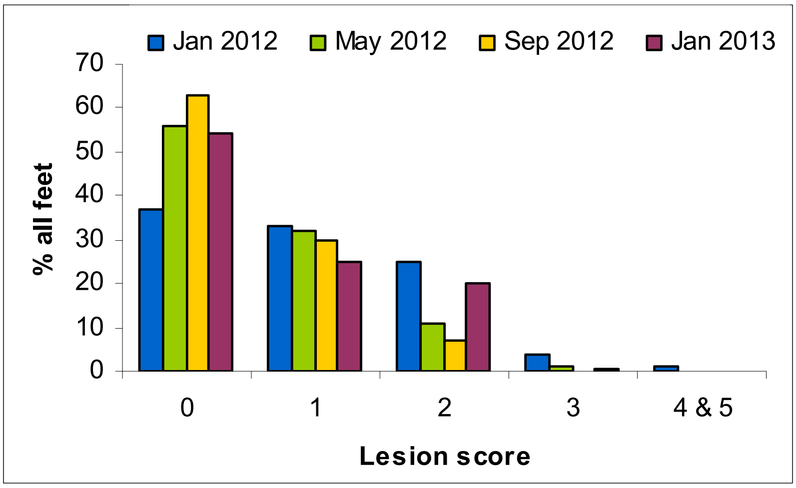


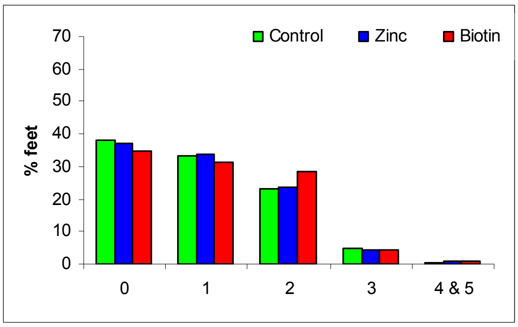
Figure 1. Distribution of white line lesion scores at each assessment visit (all treatments combined)

The proportion of sheep observed with white line lesions at the start of the study differed depending on their farm of origin. Overall the origin of 300 of the 302 ewe lambs was known and the number and percentage of animals with and without white line lesions are shown in Table 2 below. Farm A was observed to have significantly lower proportion of animals with white line lesions than the other two groups although these results need to be treated with caution as this group represented fewer than 8% of study animals.

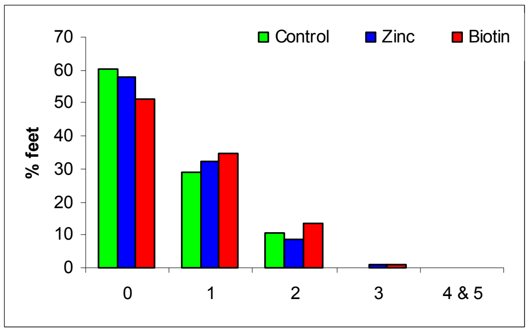
Table 2 Percentage of sheep observed with and without white line lesions at start of the study by farm of origin

	Farm A	Farm B	Farm C
Number ewe lambs in group	23	193	84
Ewe lambs without lesions	48%	9%	14%
Ewe lambs with lesions (score 1-5 in 1 or more feet)	52%	91%	86%

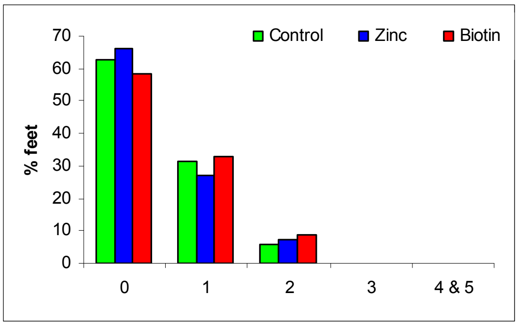
The distribution of white line lesion scores did not differ between treatments at the start of the study (Figure 2a) or at any of the subsequent assessments ($p>0.05$) (Figure 2b-2d) although the Biotin group tended to have a slightly higher proportion of feet at Score 2 at the start and this difference was maintained throughout the study. Tables detailing the breakdown of lesion scores by treatment and foot for each assessment date can be found in Appendix 2.



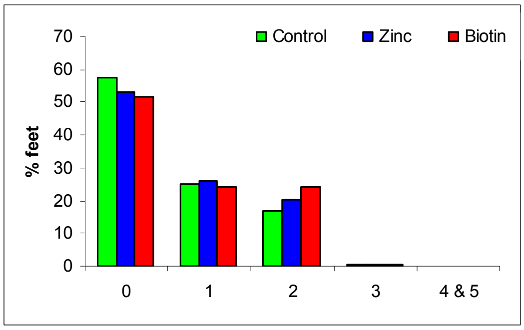
2a. January 2012 (p>0.05)



2b. May 2012 (p>0.05)



2c. September 2012 (p>0.05)



2d. January 2013 (p>0.05)

Figure 2. Distribution of white line lesions by treatment at each assessment date

A logistic regression was carried out on the data from feet where there were lesions present (scores 1-5) in January 2012 (n=763). This was used as the starting point and analyses looked at the proportion of those feet that still had lesions in May and Sept 2012, and Jan 2013, comparing the three treatments. These analyses showed no significant difference between the treatments at any of the dates (p>0.05), although there appeared to be some evidence that the proportion of front feet which still had lesions was less than for back feet in May and Sept. However this difference had disappeared by Jan 2013. Figure 3 illustrates that in feet observed with white line lesions in January 2012 there was some reduction in the percentage with lesions across all three treatment groups, although significant treatment differences were not observed.

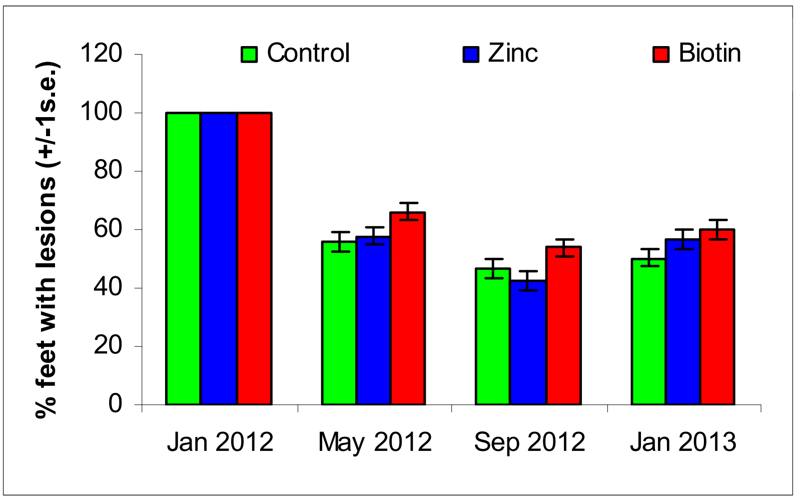


Figure 3. Feet with white line lesions (scores 1-5) at the first assessment (January 2012) and the percentage of these feet recorded with lesions at subsequent assessments

Similarly an analysis of feet with no lesions present (score 0) in January 2012 (n=445) looked at the proportion that subsequently developed lesions (Score 1-5). Overall 15% of feet were observed with lesions in May, 20% in September and 29% in January 2013. Treatment differences were not observed with 25 – 30% being observed with lesions by the end of the study. An analysis of the foot data for the 42 sheep that did not have any lesions at the start of the study highlighted that by January 2013 just under half (48%) had retained their lesion–free status throughout the whole study period and these were evenly distributed across the treatment groups.

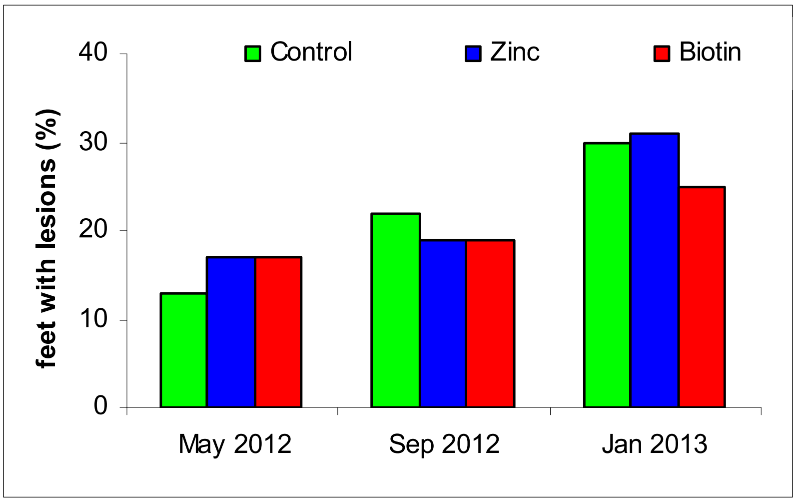


Figure 4. Feet with no lesions at the first assessment and the percentage of these recorded with lesions at subsequent assessments.

Information relating to feet that scored 2 at the start and the scores at subsequent dates can be found in Appendix 3 broken down by treatment. In addition separate figures combining all treatments that summarise the scores at each assessment date for feet that started as 0, 1, 2 or 3 can be found in Appendix 3 (Figure A3.2)

Animal performance data

Blood samples were taken to assess trace element status of sheep in January and May 2012 and January 2013. Serum zinc levels were measured to provide an indication of whether boluses were actively working in the Zinc and Biotin groups. Typically zinc levels in blood would be expected to respond within one week of bolus application. The results are summarised below in Table 3. Baseline blood samples taken in January 2012 showed that overall mean values were within the reference range for all analyses although some individuals had marginal GSH-Px levels. In May 2012 all animals were within the reference range for cobalt, copper and zinc but four out of six animals were marginal or below the recommended level for GSH-Px. Zinc levels tended to be higher in the two bolused groups and suggest that the boluses administered in January 2012 were still active in the sampled animals.
At the final assessment in January 2013 all blood samples were within the normal range for copper and had high levels of cobalt. The high cobalt levels are likely to be the result of ewes being held for a prolonged period before sampling. GSH-Px levels

across the study were on average above the recommended level but around a third of animals were marginal or below 50 µmol/l. As in May serum zinc levels tended to be higher for Zinc and Biotin ewes in January 2013.

Table 3 Trace element blood sample results

	Ref. range	Units	Overall mean	Control	Zinc	Biotin
January 2012 (n=7)						
Cobalt (Vit B12)	>188	pmol/l	617			
Copper	9-19	µmol/l	16.7			
GSH-Px	>50	U/ml RBCs	68.8			
Zinc	* see below	µmol/l	11.7			
May 2012 (n=6)						
Cobalt (Vit B12)	>188	pmol/l	702	550	634	921
Copper	9-19	µmol/l	12.4	11.6	11.7	14.0
GSH-Px	>50	U/ml RBCs	53.2	36.9	44.4	78.3
Zinc	* see below	µmol/l	21.8	19.6	21.8	24.0
January 2013 (n=15)						
Cobalt (Vit B12)	>188	pmol/l	1442	1439	1358	1530
Copper	9-19	µmol/l	14.5	14.4	14.4	14.6
GSH-Px	>50	U/ml RBCs	75.4	75.4	88.8	58.8
Zinc	* see below	µmol/l	9.6	8.9	9.3	10.6

* Ref range for zinc (serum): Marginal less than 9 µmol/l, Deficient less than 3 to 6 µmol/l

Ewe lambs/ewes were weighed at each assessment date and daily liveweight gains calculated (Figure 5 and Table 4). Analysis of the data confirmed that there were no treatment effects on ewe performance at any point during the study. Growth rates were modest throughout the study period

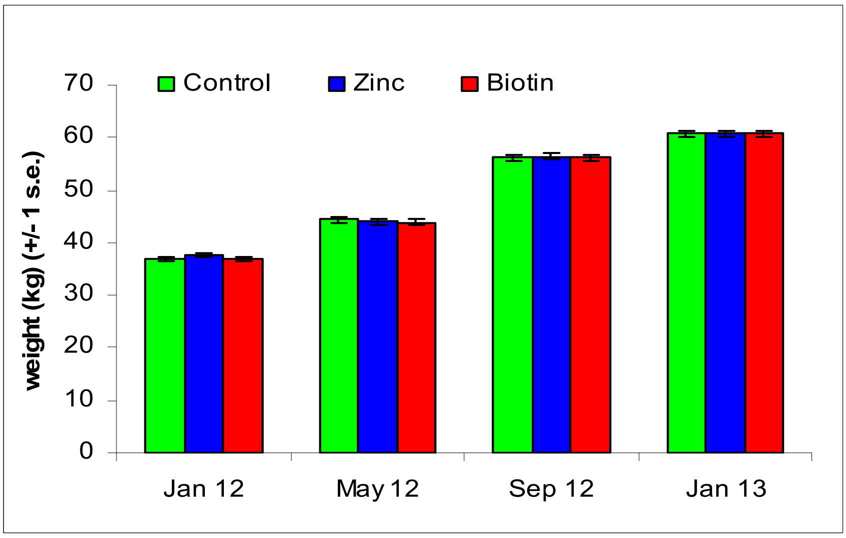


Figure 5. Effect of treatment on live weight of ewe lambs

Table 4 Live weight of ewes (kg) and growth rates (g/day)

	Control	Zinc	Biotin	s.e.d.	significance
Number of ewes at start	101	101	100		
Start weight Jan 2012	36.9	37.6	36.9	0.49	NS
Weight 11 May 2012	44.4	44.1	44.0	0.80	NS
Weight 14 Sep 2012	56.2	56.4	56.2	0.96	NS
Weight 10 Jan 2013	60.7	61.0	60.7	0.73	NS
DLWG Jan'12-May'12	74	64	69	5.7	NS
DLWG May'12-Sep'12	93	98	97	4.9	NS
DLWG Sep'12-Jan'13	39	41	39	3.1	NS
DLWG Jan'12-Jan'13	69	68	69	1.5	NS

Ewes were mated with Charollais rams in October 2012 and pregnancy scanned in January 2013. The scanning results are shown below in Table 5. Significant treatment differences were not observed.

Table 5 Pregnancy scanning results – January 2013

	Control	**Zinc**	**Biotin**	**All groups**
Number ewes at start (Jan '12)	101	101	100	302
Culled/died pre-scanning	1	2	3	6
No. ewes present at scanning	**100**	**99**	**97**	**296**
Barren (no. ewes)	0	3	1	4
Singles (no. ewes)	17	10	20	47
Twins (no. ewes)	80	82	73	235
Triplets (no. ewes)	3	4	3	10
Number lambs expected	186	186	175	547
Scanning %	**186**	**188**	**180**	**185**

Twin-bearing ewes lambed indoors from 27th February 2013 with an average lambing date of 12th March. Birth date, birth weight and sex of lambs and a note of any lambs born dead were recorded and lambs were tagged with EID tags. At birth lambs averaged 4.4 kg with weights being similar for all treatments (Table 6, Figure 6). Lamb losses at and around lambing averaged 4% across the study with little difference seen between treatments. After some time indoors in group mothering pens ewes and their lambs were turned out to grass where lambs had access to lamb creep from three weeks of age. The poor weather conditions of spring 2013 had a severe impact on study lambs and losses at grass were much higher than normal for the farm. Overall 14.7% of lambs tagged either died or were removed from the trial between tagging and 10 weeks of age. Coccidiosis was a particular concern in the trial lambs.

Lambs were weighed in May at approximately 10 weeks of age and just prior to weaning in July 2013. Performance data were analysed with 'reared' litter size as a factor in the model to allow for any differences in lamb rearing percentage between treatments. Lambs from ewes supplemented with biotin were significantly heavier at 10 weeks of age (p=0.002) and had significantly higher growth rates (p=0.003) from birth to 10 weeks (Figures 6 & 7). At this point the additional 1.9 kg of live weight achieved by Biotin lambs compared to Control lambs was worth approximately £4.60 (assuming £2.42/kg lwt). By weaning however the advantage to the Biotin treatment had disappeared with DLWG from 10 weeks to weaning and weaning weight being similar for all treatments. As lambs would have been eating significant amounts of creep feed by 10 weeks of age it is likely that this would have compensated for any longer term milk yield effects. Restricting the dataset to lambs that were reared to weaning as twins had little effect on the overall outcome. From birth to 10 weeks Biotin lambs outperformed Control lambs (similar to Zinc lambs) but performance between 10 weeks and weaning was similar for all treatments.

Table 6 Lamb performance birth to weaning

	Control	Zinc	Biotin	s.e.d.	significance
Number of lambs tagged	149	157	137		
Birth weight (kg)	4.4	4.3	4.4	0.09	NS
10 week weight (kg)	21.8	22.3	23.7	0.56	*p* = 0.002
Weaning weight (kg)	35.7	35.8	36.7	0.74	NS
DLWG birth to 10 wks (g)	245	251	269	7.2	*p* = 0.003
DLWG 10wks to weaning (g)	257	253	246	8.4	NS
DLWG birth – weaning (g)	253	253	259	5.7	NS

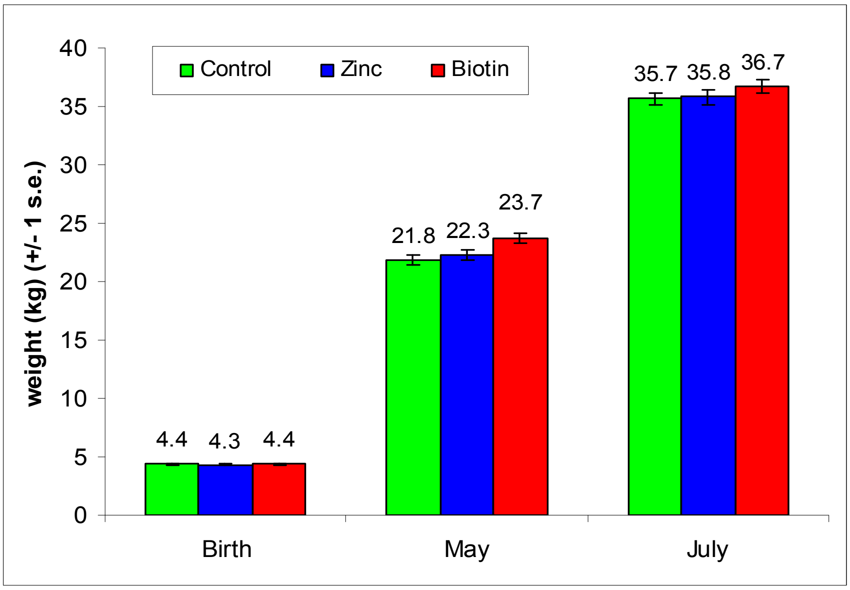


Figure 6. Lamb live weight at birth, 10 weeks and weaning

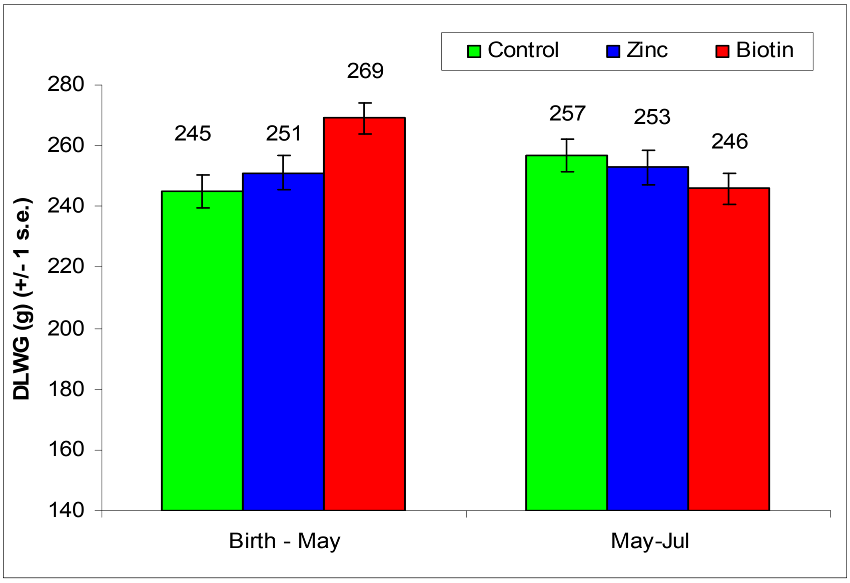


Figure 7. Lamb growth rates birth to 10 weeks and 10 weeks to weaning

Conclusions

This study identified that compared to control animals, four-monthly oral supplementation with a bolus releasing 82 mg/day available zinc only or 5mg/day biotin and 82 mg/day zinc did not have a significant effect on the severity of WL scores.

Biotin did not appear to facilitate healing of white line lesions or protect sheep against white line disease in this study.

As the study was conducted in a single lowland flock of replacement ewe lambs with relatively mild lesions it may be useful to evaluate the effect of biotin in a larger population with more severe lesions over a longer study period.

The effect of environmental and climatic conditions and the role of genetics in WL of sheep also warrant further research. The weather was particularly wet in 2012 -13 and this may have had an impact on the effect of biotin on white line lesions. Significant differences in the level of white line disease were seen between sheep sourced from different farms in this study indicating that environmental or genetic factors may play an important part.

The cost of a biotin bolus was estimated at around £1.60/head per dosing occasion equating to a cost of around £6.40 for this trial. However if the study had been solely looking at lamb growth rates a single bolus administered in January would have been adequate.

Supplementing ewes with biotin had a significant positive effect on lamb performance between birth and 10 weeks of age. Overall live weight at 10 weeks and DLWG to 10 weeks were lifted by 8% compared to the Control and Zinc treatments. Birth weight was unaffected by treatment. At 10 weeks the financial benefit to the Biotin treatment was estimated to be £4.60 /hd assuming liveweight price of 242 ppkg.

Performance to weaning was similar for all treatments but is likely to have been influenced by creep feeding of lambs. It may be beneficial to evaluate the effect of biotin on lamb performance in a flock where lambs do not receive creep feed. Greater dependence on creep feed rather than milk may explain this levelling off in performance across treatments by weaning but the results do indicate superior performance in lambs born to ewes that received biotin which is likely to have been due to higher milk yield in the first 10 weeks of lactation.

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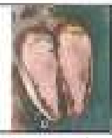
Appendix 1. White line lesion scoring scale

WHITE LINE LESION SCORING SCALE

Score	Description of score	Example
0	**No white line lesion observed**	
1	**Minor separation of the white line ("shelly hoof")** Separation of the white line along less than half the length of the foot and to less than half way up the wall	
2	**Moderate separation of the white line** Separation of the area of white line with more than half the length of foot affected and extending up more than half way up wall; may appear as a half-moon appearance with no evidence of impaction or infection of the laminae	 
3	**Major separation of the white line** Separation of the white line along much of length and height of wall, with or without loss of the overlying wall. This score includes lesions which are impacted with dirt, mud or faeces	 
4	**Discrete lesions in the white line with no separation** Black or discoloured spots anywhere on the sole in the region of the white line. There is no separation of the hoof wall from the underlying laminae	 
5	**Active infection of the white line** Purulent discharge from the white line area and/or sinus tracts extending from the white line to coronary band with heat and swelling of foot and/or purulent discharge at the coronary band	 
6	**Healed white line lesion** Dry, intact laminae covered by an outer keratinised hoof with no separation. The outer wall may flake or lift off over the area of a previous but now healed white line lesion	

Appendix 2. Lesion score summary tables

Day 0 Jan 30th - 31st 2012 (FL = front left, FR = front right, BL = back left, BR = back right)

Group 1 Red (number)

Score	**FL**	**FR**	**BL**	**BR**	**Total**	**%**
0 (none)	42	41	25	32	140	35.0
1 (minor)	29	28	38	31	126	31.5
2 (mod)	24	23	33	34	114	28.5
3 (major)	5	6	3	3	17	4.3
4 (discrete)	0	2	0	0	2	0.5
5 (active inf)	0	0	1	0	1	0.3
6 (healed)	0	0	0	0	0	0.0
Total	100	100	100	100	400	

Group 2 Blue (number)

Score	**FL**	**FR**	**BL**	**BR**	**Total**	**%**
0	50	39	29	33	151	37.4
1	28	38	38	32	136	33.7
2	20	19	28	29	96	23.8
3	2	3	6	6	17	4.2
4	1	2	0	1	4	1.0
5	0	0	0	0	0	0.0
6	0	0	0	0	0	0.0
Total	101	101	101	101	404	

Group 3 Green – Control (number)

Score	**FL**	**FR**	**BL**	**BR**	**Total**	**%**
0	56	44	25	29	154	38.1
1	22	29	40	43	134	33.2
2	18	23	32	20	93	23.0
3	4	4	4	8	20	5.0
4	1	1	0	0	2	0.5
5	0	0	0	1	1	0.2
6	0	0	0	0	0	0.0
Total	101	101	101	101	404	

May 11th 2012

Group 1 Red

Score	FL	FR	BL	BR	Total	%
0	42	48	33	40	163	41.2
1	28	36	39	34	137	34.6
2	12	6	18	17	53	13.4
3	1	0	2	1	4	1.0
4	0	0	0	0	0	0.0
5	0	0	0	0	0	0.0
6	16	9	7	7	39	9.8
Total	99	99	99	99	396	

Group 2 Blue

Score	FL	FR	BL	BR	Total	%
0	62	58	46	50	216	53.5
1	20	32	42	36	130	32.2
2	8	8	10	10	36	8.9
3	2	0	1	2	5	1.2
4	0	0	0	0	0	0.0
5	0	0	0	0	0	0.0
6	9	3	2	3	17	4.2
Total	101	101	101	101	404	

Group 3 Green - Control

Score	FL	FR	BL	BR	Total	%
0	59	57	45	41	202	50.0
1	25	23	31	38	117	29.0
2	5	9	17	12	43	10.6
3	0	0	0	0	0	0.0
4	0	0	0	0	0	0.0
5	0	0	0	0	0	0.0
6	12	12	8	10	42	10.4
Total	101	101	101	101	404	

September 14th 2012

Group 1 Red

Score	FL	FR	BL	BR	Total	%
0	66	54	39	36	195	50.3
1	18	24	43	43	128	33.0
2	7	8	10	8	33	8.5
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	6	11	5	10	32	8.2
Total	97	97	97	97	388	

Group 2 Blue

Score	FL	FR	BL	BR	Total	%
0	71	63	55	45	234	58.5
1	15	21	33	38	107	26.8
2	5	7	7	9	28	7.0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	9	9	5	8	31	7.8
Total	100	100	100	100	400	

Group 3 Green - Control

Score	FL	FR	BL	BR	Total	%
0	68	65	46	41	220	54.5
1	25	21	37	44	127	31.4
2	3	4	10	7	24	5.9
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	5	11	8	9	33	8.2
Total	101	101	101	101	404	

January 10th 2013

Group 1 Red

Score	FL	FR	BL	BR	Total	%
0	46	50	48	45	189	49.2
1	25	16	31	20	92	24.0
2	22	25	17	28	92	24.0
3	0	0	0	1	1	0.3
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	3	5	0	2	10	2.5
Total	96	96	96	96	384	

Group 2 Blue

Score	FL	FR	BL	BR	Total	%
0	51	42	55	50	198	51.6
1	21	27	24	28	100	26.0
2	21	22	17	18	78	20.3
3	1	1	0	0	2	0.5
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	2	4	0	0	6	1.6
Total	96	96	96	96	384	

Group 3 Green - Control

Score	FL	FR	BL	BR	Total	%
0	53	51	57	61	222	55.5
1	25	26	29	20	100	25.0
2	19	17	14	18	68	17.0
3	0	2	0	0	2	0.5
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	3	4	0	0	8	2.0
Total	100	100	100	100	400	

Appendix 3 Additional lesion score data

Table A3.1 Feet scoring 2 at start and the proportions falling into each category at subsequent dates

	No. at start	Jan 12 (%)	May 2012 (%)				September 2012 (%)				January 2013 (%)			
Lesion score		**2**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Control	93	100	42	39	19	0	52	35	13	0	45	32	23	0
Zinc	96	100	28	47	21	4	48	38	14	0	38	29	32	1
Biotin	114	100	29	46	23	2	40	45	15	0	37	25	38	0

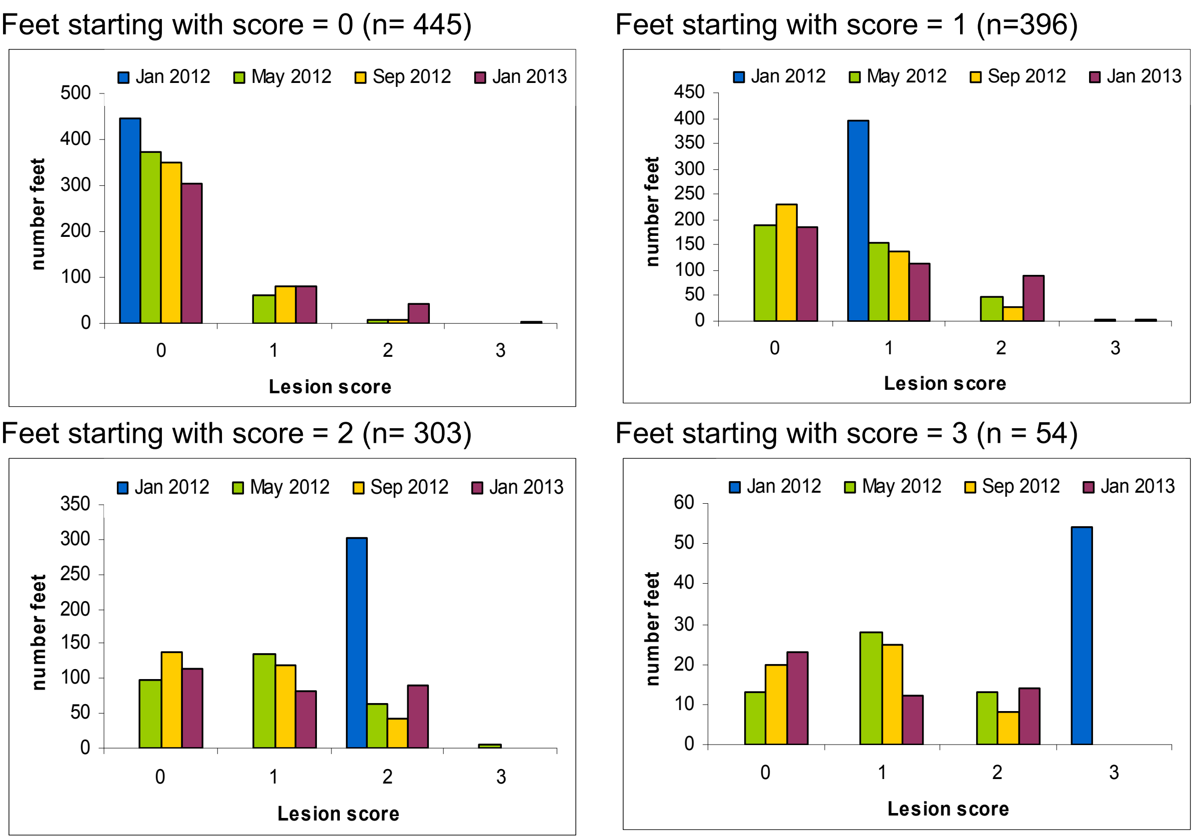


Figure A3.2 Feet with white line lesions (scores 0-3) at the first assessment (January 2012) and the scores at subsequent assessments (all treatments combined)