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Evangelia N. Sossidou

► To cite this version:

Evangelia N. Sossidou. Different soil media for free-range laying hens. British Poultry Science, 2008, 49 (04), pp.390-395. 10.1080/00071660802159041 . hal-00545336

HAL Id: hal-00545336

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Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2007-272.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	10-Feb-2008
Complete List of Authors:	Sossidou, Evangelia; NATIONAL AGRICULTURAL RESEARCH FOUNDATION, Animal Research Institute; NATIONAL AGRICULTURAL RESEARCH FOUNDATION, Veterinary Research Institute
Keywords:	Behaviour, Free range, Environment

Revised to END OF REFERENCES

E. N. SOSSIDOU, S. P. ROSE¹, S. S. P. SILVA¹, N. W. HALL²
A. TSERVENI-GOUSSI³ AND V. CHRISTODOULOU

¹National Agricultural Research Foundation (N.A.G.R.E.F.), Animal Research Institute,
58100 Giannitsa, Greece; ²The National Institute of Poultry Husbandry, Harper
Adams University College, Edmond, Newport, Shropshire, England, TF10 8NB,
³Crop and Environment Research Centre, Harper Adams University College,
⁴Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, Department of
Animal Production, Ichthyology, Ecology and Protection of Environment, 54124
Thessaloniki, Greece

Running head: SOIL FOR FREE-RANGE LAYERS

Correspondence to

Accepted 19 February 2008

Abstract.

1. A replicated experiment compared bird use, soil structure, grass wear and free-moving nematode populations in 4 different soil media (recycled vegetable compost, 90% recycled vegetable compost and 10% sand, re-used topsoil and sterilised topsoil) all with established grass swards within the range area of a large free-range laying hen unit.
2. The birds initially spent a greater proportion of their time on the two topsoil swards in comparison to the two compost-based swards. However, once the whole flock of hens had prolonged access to the different swards (unfenced areas) there were no significant differences in the number of birds that frequented the different sward types.
3. The two compost-based soil media had 33% fewer nematodes per gram of dry soil compared to the two topsoil based media. However, the rate of loss of grass from the subplots was greater with the two compost-based soil media; this was probably due to the greater porosity of these types of soil media.

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Corresponding author. Dr Evangelia N. Sossidou, National Agricultural Research Foundation, Animal Research Institute, 58100 Giannitsa, Greece. Tel.: +30 23820 31700; Fax: +30 23820 32332; E-mail: sossidou.arig@nagref.gr¶
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INTRODUCTION

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There is a requirement that free-range laying hens must have continuous day-time access to open-air runs that are mainly covered with vegetation (EC Council Directive 199/74/EC). Permanent, or semi-permanent, housing positioned within grass-sown leys are often the most practical method of providing these conditions for commercial laying hen flocks. A good-quality sward is important in that it provides some forage for the birds at range and it avoids the area becoming muddy. Mud can stick to the feet of the birds and so can transfer inside the egg laying areas. Even with a good quality sward, the areas close to the entrances of houses frequently become denuded.

Parasitic worm infections are a particular problem in un-caged laying hens, and there is a high incidence in commercial free-range poultry units (Pennycott and Steel, 2001). The parasites are mostly nematode worms and their incidence is higher than in other loose-housing systems (Permin *et al.*, 1999). The nematode worms survive in the soil and this allows them to complete their life-cycle successfully. There is a need to examine if particular soil media can be used to reduce the surviving nematode population in the land surrounding free-range laying houses.

The EU Waste Management Strategy (COM(96) 399) has resulted in the Landfill Directive 99/31/EC that requires 25% of household waste to be composted. Composted recycled vegetable waste is now readily available and inexpensive. Not only does the composting process eradicate nematodes from the vegetable material (Noble and Roberts, 2004) but also the stable composted material reduces the ability of nematodes, and some bacteria, to repopulate (USEPA, 1997). Vegetable waste compost can be used directly, or with some modification, as a soil medium and good grass swards can be established.

Although the use of vegetable waste compost as a soil medium for the whole range area would be uneconomic, it is possible that the managers of commercial egg production units would consider using it to seed areas that are close to the entrances of the fixed housing and that probably have the greatest bird use. There is a need to examine whether the use of recycled vegetable compost has the potential to be used as a soil medium in the range areas and whether its use is practicable. The compost has a high water-holding capacity, so it may be that compost that includes some free-draining material, for example sand, may improve its characteristics for use in free-range units.

The objective of this study was to give preliminary information on the potential value of composted recycled vegetable waste as a soil medium for commercial free-range units.

MATERIALS AND METHODS

The experimental work was conducted at Harper Adams University College using a 4500 bird free-range egg production unit. The unit was comprised of a fixed, environmentally-controlled laying hen house that contained nest boxes, drinking and feeding equipment and 380 m² of slatted floors. The building had a total of 9 m of external pop-holes, that gave the birds access to a slatted, partially-enclosed verandah area (80 m²). Pop-holes (9 m in total) gave the birds access from the verandah to the range area. The fixed unit was sited centrally in an approximately square 2.0 hectare field. The unit had been in continuous production for the 9 years since its establishment and no amendments to the range had been made during this time. The range area used for the unit was sown to grass and the soil type was a free-draining sandy loam over sandstone. A flock of sheep was allowed to graze the range area but

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their access was restricted to more than 100 m from the fixed laying hen house and away from the three experimental blocks.

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Three experimental areas (with a dimension of 4 m x 4 m) were chosen that were each approximately 50 m from a verandah entrance to the laying hen house.

Each area was further subdivided into 4 subplots each of 4 m² (dimensions of 4 x 1 m) and 20 cm of the topsoil was removed and replaced with one of the 4 soil media.

These media were either recycled composted vegetable waste, a mixture of 90%

recycled composted vegetable waste and 10% sand, part-sterilised (steam application

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to a soil temperature of 86°C for approximately 90 min) topsoil or un-sterilised topsoil

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obtained from the same site. The relative positions of each of the soil media subplots within the main plots were chosen using random numbers. A proprietary grass seed mixture (a mixture of grass varieties that included 35% ryegrass and 35% red fescue) was used to seed each of the 4 different soil media.

The grass swards were sown in March 2005 and a new 16-week-old flock of

Lohmann Brown laying hens were placed in the house in the following month. The

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flock was managed according to the established rules for free-range egg production

and the birds were fed, ad libitum, a proprietary laying hen feed. Shade and water was

provided on the range at locations that were approximately 25 m from each of the

experimental plots but feed was only provided within the fixed laying house. The

experimental swards were protected from access by the birds by wire cages until

September 2005 to allow correct establishment of the sward. During this time, the

grass was cut mechanically and removed and grass length was maintained at 5 cm + 2

cm throughout this period. No fertiliser or chemical applications were made to the

grass during this time.

Behavioural observations

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110 Observational data of the laying hens were collected from the established plots during
111 September 2005 using the general procedures outlined by Martin and Bateson (1993).
112 The first objective was to determine the relative preference of the hens for the swards
113 grown on the 4 soil media. This work was undertaken immediately prior to allowing
114 the flock access to the experimental plots. Each plot was securely fenced and 12
115 randomly-selected birds were placed into each plot where they had free access to each
116 of the 4 subplots. Observations of the location of the birds placed within the plot were
117 taken for one hour. After this time the birds were removed and returned to the main
118 flock and a further 12 birds were randomly selected and placed in the same plot for
119 another hour of observations. Observations of bird location were therefore made on
120 two occasions (morning and afternoon) for each single main plot and observations
121 were made on each of the three main plots on following days of the same week.

122 The second objective of the behavioural observations was to determine the
123 relative use of the swards by the entire flock. The plot areas were previously unfenced
124 and the whole flock had been allowed free access to the three experimental areas as
125 well as the rest of the whole range area. Two 1-h observational periods counted the
126 numbers of birds standing on each of the 4 subplots for each open area.

127 **Soil structure and grass cover measurements**

128 After the first (fenced) behavioural observations, the three plots were left unfenced in
129 ambient conditions and the free-range flock had daily access to the three plots, and
130 any other area of the range area. Six series of measurements were made at the
131 beginning of the each month, from October 2005 to March 2006 to quantify the
132 amount of grass cover remaining on the soil media in each plot. Grass score was
133 described as the proportion of grass vegetation that covered each subplot area. A

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weather station at Harper Adams University College was used to collect monthly rainfall data during the experimental period.

Two methods were used to assess soil structure on samples taken in October 2006 after approximately one month of continuous use by the flock but when there was still a substantial amount of grass cover on each of the subplots. The first method measured penetrometer resistance with a 30° cone penetrometer (Finlay Irvine Ltd, Penicuik, Scotland) using the method described by USDA (1995). Two series of readings were taken – one at the surface of the soil and the second 10 cm below the surface. Ten readings were taken for each subplot and the mean computed. The second method measured the porosity of the soil using thin-section microscopy. An undisturbed sample (5 cm depth) was taken from each subplot. Thin sections were prepared using the method of Murphy (1986) with image capture (Coolview II, Photonic Science Ltd., Mountfield, UK) and electronic image analysis (Image Pro Plus, Media Cybernetics Inc., Silver Spring, USA)

Extraction of free living nematode stages used an adapted Baermann funnel method (Hooper 1986). An approximate weight of 600 g of soil was sampled from the top 2 cm of the soil surface from each subplot. Free moving nematodes were extracted by soaking the soil sample for 12 h in water and allowing the nematodes to move into the aqueous phase. The nematodes were extracted into 20 ml of water and, after thorough mixing, a count of motile nematodes was made on a 1 ml sub-sample using a 10 x10 magnification.

Statistical analysis

Treatment differences were statistically compared using a randomised block analysis of variance. The treatment sums of squares were orthogonally partitioned into three specific pre-planned treatment comparisons (Steel and Torrie, 1960). All statements

of significance are based on a probability of less than 0.05. Statistical analysis was performed using SPSS (1997) and GENSTAT software.

RESULTS

All the grass swards established successfully in each of the 4 soil media on each of the three separate areas and there was complete grass cover on each of the subplots when the wire cage protections were removed and the birds were given free access. There were no visual differences in the condition or growth of the swards between the 4 soil media. The monthly rainfall during the experimental period for October 2006 to March 2006 was 80.4, 67.4, 38.8, 12.8, 27.2 and 54.0 mm respectively, compared to the mean rainfall in the same month over the previous 10 years of 65.3, 57.7, 54.9, 37.0, 42.2 and 39.5 mm respectively. Although there were monthly variations, the total rainfall over the 6-month period (280.6 mm) was similar to the mean 10-year average rainfall for these months (296.6 mm) at the research site.

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There were statistically significant differences in the hens' preferences for grass swards grown on the 4 soil media when the birds were first introduced to the 4 different swards (fenced areas) (Table 1). The birds spent a greater proportion ($P<0.01$) of their time on the two topsoil swards in comparison to the two compost-based swards. However, once the whole flock of hens had prolonged access to the different swards (unfenced areas) there were no significant ($P>0.05$) differences in the number of birds that frequented the different sward types.

In common with the rest of the range area that was adjacent to the three main plots, the grass sward gradually disappeared over the course of the 6-month period. At the end of the 6-month period, no subplot still had any grass cover (Figure 1). However, the rate of loss of grass from the subplots was greater ($P<0.001$) with the

184 two compost-based soil media and the rate of loss in the 100% compost soil was
185 greater ($P<0.05$) than the 90% compost and 10% sand.

186 Results on the effect of the 4 soil media on soil structure and free-moving
187 nematodes are presented in Table 2. Porosity values were generally satisfactory for
188 air and water movement and root development (Yilmaz *et al*, 2003). The 100%
189 Compost had a very high porosity rating at a proportion of 0.423 (pores larger than
190 0.02 mm in diameter). The 100% compost had a greater ($P<0.05$) porosity than the
191 other three soil media and with such a high porosity and organic matter content, a soil
192 that was weak to excavation would be expected. Penetrometer resistance values are
193 generally low to very low (Yilmaz *et al*, 2003). Although there were no ($P>0.05$)
194 differences at the surface, the penetrometer resistance at 10 cm was greater ($P<0.01$)
195 in the 90% compost + 10% sand treatment compared to the 100% compost soil
196 medium (Table 2). The two compost-based soil media had 33% fewer ($P<0.05$)
197 nematodes per gram of dry soil compared to the two topsoil based media.

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DISCUSSION

200 When birds were first introduced to the fenced areas they preferred to move to the
201 topsoil swards in comparison to the swards grown on compost-based soils. The birds
202 used in this experiment had had a long experience of ranging on grass grown on
203 topsoil and evidently had an initial aversion to the recycled vegetable waste swards.
204 Birds are known to use olfactory cues to locate and discriminate between foods
205 (Wenzel and Sieck, 1972) and were apparently able to detect the difference between
206 the two soil media types. However, the lack of differences in preferences in the
207 unfenced areas, when the birds were allowed long-term access to the areas, indicated

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208 that these initial preferences were soon overcome and other cues to the birds' location
209 within the range dominated their preferences.
210 Approximately 6 birds/h stood on each of the experimental plots during the
211 behavioural observation periods. This was a relatively high use of the plots by the
212 laying hens in comparison to adjacent parts of the range area. The three plots only
213 comprised approximately 0.2% of the total range area and not all birds made any use
214 of the range. Zeltner *et al.* (2004) observed a 3000 bird free-range flock and found
215 that only 19.5% of the birds used the range at all and over half of these never went
216 away from the area immediately nearest to the fixed house.

217 The faster rate of loss of grass from the compost-based soils would be of great
218 practical significance to a commercial free-range egg producer who would want to
219 maintain a good grass cover on the range. There was no evidence that the birds
220 preferred the compost-based soil swards, but the faster rate of grass loss may have
221 been due to a poorer soil structure. The high porosity of the compost-based soils
222 indicates that the soil was more fragile and liable to break up under the feet of the
223 birds as seen in the grass cover results. The 90% compost and 10% sand treatment
224 gave a significant ($P<0.05$) reduction in porosity. However porosity was still
225 satisfactory for air and water movement and root development (Yilmaz *et al.*, 2003),
226 although it was not as high as the two topsoil treatments. The penetrometer results for
227 10 cm depth are interpreted as showing that soil just below the surface provided better
228 support for the overlying soil aiding sward preservation. This indicates that particular
229 soil media formulations, that are based on compost, could be derived that have
230 suitable soil structure characteristics.
231 The nematode quantification technique measured total motile nematodes in the soil
232 and was not able to identify between species. However, it gives an indication of the

probable poultry nematode worm infestation on the different swards. The mean size and number of nematodes was used to approximately calculate the motile nematode biomass and this indicated that it was approximately 400 mg per m² of soil media. This is similar to the mean motile nematode biomass found in a survey of UK temporary grass swards (Cook *et al.*, 1992). The part-sterilised topsoil had the numerically highest ($P>0.05$) nematode population of the 4 soil media. The soil nematode sample was taken approximately 6 months after the soil media was introduced and this indicates that nematodes are quickly able to repopulate an area. The two compost-based soils had fewer ($P<0.05$) nematodes than the topsoil-based soils and this indicates that there are soil media that can slow the increase in nematode populations in the range areas of laying hens. Only two rudimentary compost formulations were used in this experiment, but it shows that the inclusion of a proportion of composted vegetable waste could have significant long-term benefits to the health status of free-range laying hen flocks. The results therefore indicate that there may be potential to use compost to formulate soil media to reduce the nematode worm populations in the range areas for laying hens.

Acknowledgements

The authors acknowledge the support of the British Council and the National Agricultural Research Foundation through the project “*Improvement of the welfare of free-range laying hens by innovative changes in range management*”.

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Deleted: In conclusion, this study has given preliminary evidence that composted vegetable waste can be used as a soil media for free-range hens. The high porosity of the 100% material resulted in a rapid loss of grass but modifications of the media formulation could improve this characteristic significantly. Although birds had an initial aversion to use of grass swards grown on compost, this was quickly overcome and the reduction in nematode populations after six months of use indicated that there could be potential health and productive performance advantages from the use of these types of soil media.¶

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Table 1. *Hens' preferences for grass swards grown on the 4 soil media.*

Soil media	Enclosed area study (Proportion of time spent standing on treatment subplot)	Open area study Number of birds (from the whole flock) standing on the subplot (No. birds/h)
Composted vegetable waste	0.175	6.44
90% Composted vegetable waste + 10% sand	0.135	6.86
Topsoil	0.288*	7.94
Sterilized topsoil	0.402*	5.58
SED ¹ (6 degrees of freedom)	0.04547	1.447

* = Factors within the same coloumn that are significantly different at $P=0.05$
SED¹=Standard Errors of Differences of Means.

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Table 2. Effect of *the 4* soil media on soil structure and *grass cover measurements*.

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Variable	Soil media				
Soil penetrometer resistance (Cone index units)	Composted vegetable waste	90% composted vegetable waste 10% sand	Topsoil	Sterilised topsoil	SED ¹ (6 degrees of freedom)
(Sampled in October 2005)					
Surface	18.0	24.1	23.9	27.1	5.62
10 cm depth	30.0	38.8**	26.1	33.7	3.76
Soil microscopy (Sampled in October 2005)					
No. of pores per 50 mm ²	424	351	445	384	67.8
Porosity (Proportion of soil as air for pores greater than 0.02 mm diameter)	0.423*	0.204	0.278	0.197	0.0204
Soil nematode count (Sampled in February 2006)					
Water content of soil sample (g/kg)	306	268	262	302	52.5
Nematode count per g of dry matter	13.8*	14.2*	18.6	23.5	3.93

* = Factors within the same row that are significantly different at $P=0.05$.** = Factors within the same row that are significantly different at $P=0.01$.SED¹ = Standard Errors of Differences of Means.

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Figure 1. Proportion of grass cover on four soil media (vertical bars indicate SED (6df))