Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

# Effect of range use on endo- and ectoparasite infestation in italian organic egg production

Susanna Lolli, Guido Grilli, Lorenzo Ferrari, Paolo Ferrari & Valentina Ferrante

**To cite this article:** Susanna Lolli, Guido Grilli, Lorenzo Ferrari, Paolo Ferrari & Valentina Ferrante (2019): Effect of range use on endo- and ectoparasite infestation in italian organic egg production, Italian Journal of Animal Science, DOI: 10.1080/1828051X.2018.1564377

**To link to this article:** https://doi.org/10.1080/1828051X.2018.1564377

ITALIAN JOURNAL OF ANIMAL SCIENCE
https://doi.org/10.1080/1828051X.2018.1564377

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION



# Effect of range use on endo- and ectoparasite infestation in italian organic egg production

Susanna Lolli[a], Guido Grilli[b], Lorenzo Ferrari[a], Paolo Ferrari[c] and Valentina Ferrante[a]

[a]Dipartimento di Scienze e politiche ambientali, Università degli Studi di Milano, Milano, Italy; [b]Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Milano, Italy; [c]Centro Ricerche Produzioni Animali, Reggio Emilia, Italy

**ABSTRACT**

In organic farms, hens can freely access the outdoor areas, a potential source of helminth infections. This study aimed to evaluate the effective use of free-range access in organic laying hen's farms located in Italy. The other objective was to quantify the major risk factors for endo- and ectoparasite infestation and its effect on performance, health and welfare, correlated to the use of free-range access. Fifteen organic farms of laying hens were assessed and visited twice. The total number of hens in the three zones was counted and recorded three times daily. Indirect indicators (characteristics of vegetation cover, amount and weight of droppings) were recorded. To evaluate parasite infestation, 15 fresh faecal samples per flock were collected from the ground/floor or perches. From seven farms, 15 gastrointestinal tracts from end-of-lay hens were qualitatively and quantitatively examined for the presence of endoparasites at different developmental stages. The percentage of hens observed outdoors in zone 3 was affected by free-range features. As expected, zone 1 was more frequented by hens than the others. The correlations among number and weight of droppings collected outside, vegetation score and the percentage of hens showed interesting results. Only three species of parasites were found during faecal samples analysis. There was a significantly and positively correlation between the number of Capillaria and Ascaridia eggs. The results showed that the presence in the free-range area of mounds, trees and bushes attracted more animals outside and allowed them to show their natural behaviour.

**HIGHLIGHTS**

- Consumer's motivations to buy organic food are related to human health, environmental concerns and animal welfare. The higher animal welfare standards of organic production lead consumers to buy organic instead of conventional food of animal origin. However, some health and welfare problems that could affect organic egg production need to be addressed. The characteristics of the outdoor range are one of the factors that can most affect its use. Moreover, the use of the free range is considered one of the major risk factors for endo- and ectoparasite infestation in organic layers that can affect performance, health and welfare.

**ARTICLE HISTORY**
Received 18 July 2018
Revised 6 December 2018
Accepted 23 December 2018

**KEYWORDS**
Organic egg production; welfare indicators; laying hens; free-range use; parasites

## Introduction

In 2016, Italian organic agriculture spanned over 1,492,579 hectares, with an increase of 7.5% compared to 2015 (SINAB 2016). Organic crops account for 12% of the national utilised agricultural area (UAA), which is growing steadily by almost one percentage point per year. Furthermore, organic farms account for 3.6% of total farms.

The livestock sector has also seen some development in our country over the years.

Organic egg production in Italy is the largest animal production market, with an increase over the previous year of 5.7% (BIOREPORT 2016).

Council Regulation (EC) No. 834/2007 set the maximum indoor and outdoor stocking densities to guarantee animal comfort and welfare at 6 hens/m$^2$ indoors, 4 m$^2$/hen outdoors. The enrichment of the outdoor range with trees and shaded areas may encourage hens to use and explore their surroundings by reducing the fear of predators. Many studies have shown a positive

CONTACT Dr Valentina Ferrante valentina.ferrante@unimi.it Department of Environmental Science and Policy Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

relationship between the availability of overhead cover and the % of hens in the range (Hegelund et al. 2002; Zeltner and Hirt 2003). Free-range egg farmers are encouraged to provide pasture, shaded areas and shelterbelts to attract birds into free-range areas (Nagle and Glatz 2012). Shade and shelter in the range area offer better welfare, considering that hens may perform their species-specific ethogram, like dust bathing and scratching. The restricted use in organic farming of anthelminthics and antiparasiticides makes controlling parasitic infections more difficult. Heavy loads of external and intestinal parasites can pose health implications for the hens, such as impaired weight gain and growth, decreased egg production, increased mortality, and possibly anaemia (Permin et al. 2006; Bennet et al. 2011). Endoparasite infestation is widespread in organic poultry production, and it is a health and welfare issue. Permin et al. (1999) found the presence of *Ascaridia galli* (roundworm) and *Heterakis sp.* (caecal worm) in approximately 64% and 73% of organic hens, respectively. European studies have reported the presence of mixed helminthic infections from *Ascaridia*, *Heterakis* and *Capillaria* (Pennycott and Steel 2001; Bestman and Wagenaar 2003; Kaufmann et al. 2011; Thapa et al. 2015). Organic farming systems offer favourable conditions for endoparasites because their eggs can accumulate in the environment, remaining infectious for a year or more (Farr 1956). In farmed poultry species, there are several ectoparasites, both insects and arachnids, like lice, fleas and mites. Red mite (*Dermanyssus gallinae*) is currently one of the most detrimental ectoparasites in laying birds across several countries. Symptoms of *D. gallinae* infestation include a reduction in production, poor egg quality, increased mortality and a compromise to welfare (Mul et al. 2009). The red mite spends the majority of its short life-cycle hidden deep within the house substructure, feeding on its host for only short periods of time. For this reason, red mites occupy free-range or barn systems, since a greater number of potential hiding places are available (Arkle et al. 2005).

This study aimed to evaluate the effective use of free-range areas in organic laying hen's farms located in Italy. The other objective was to quantify major risk factors for endo- and ectoparasite infestation in organic layers and its effect on performance, health and welfare, correlated to the use of free-range areas.

## Materials and methods

In the present study, 15 organic farms (north-east of Italy) of laying hens were assessed. The flock's sizes ranged from 420 to 3000 hens.

Each flock was visited twice: the first visit was at the peak of lay, between 30 and 40 weeks of age; the second was at the end of lay, at 1 or 2 weeks before slaughter (from 60 to 70 weeks of age).

### Questionnaire

Management data were collected by a questionnaire to the farmers that included the following sections:

- System and flock description
- Management of free-range areas
- Feeding and health management
- Performance

### Use of free-range areas

Each visit started by dividing each free-range area into three zones: 0–20 m, 20–40 m and more than 40 m away from the hen house and by numbering them from 1 to 3. In nine farms, a veranda was present; in this case, the number of hens was also counted there. Additional information about the provision of natural and/or artificial enrichment in every zone was recorded in a diagram (i.e. trees, bushes, hedges, drinkers, and artificial covers). The total number of hens in each zone was counted and recorded three times daily during the visits.

Indirect indicators, such as the characteristics of vegetation cover, amount and weight of droppings at pre-defined locations across the free-range area, were recorded.

The vegetation covers of the whole free-range area have been categorised according to the type (e.g. grass, plants or trees) and the amount of cover (score from 1 to 5, where 1 = 0–20% 2 = 21–40% 3 = 41–60% 4 = 61–80% 5 = 81–100%). Manure excretion was assessed by recording the number and the weight of droppings found in predefined sample areas (1 $m^2$).

### Parasites

To evaluate parasite infestation, fifteen fresh faecal samples per flock were collected from the ground/floor or perches. The samples were of intestinal dropping and not droppings from *caeca*. The samples were examined individually according to the McMaster technique (Permin et al. 1999). The sensitivity of this method is 50 eggs per gram of faeces.

From seven farms, 15 gastrointestinal tracts from end-of-lay hens were qualitatively and quantitatively examined for the presence of endoparasites at

different developmental stages. The hens were fasted approximately 6 hours before slaughter to avoid too much content in the intestines. The gastrointestinal tracts were refrigerated (5 °C) and examined within 48 hours. The following sections of the gastrointestinal tract were opened: proventriculus, small intestine, *caeca* and *rectum*. All macroscopically visible *A. galli* worms were collected and divided into two categories according to their size (1–2.5 cm small immature worms; above 2.5 cm adult worms).

Cestodes are collected and scored as no cestodes (0), one cestode (1), two to five (2), and more than five (3).

The *caeca* were examined for the presence of *Heterakis gallinarum*. The mucosal surface of the intestine was inspected for the presence of *Capillaria*.

The presence of red mites was quantified using 10 traps during each visit. The traps were fixed on the underside of the cross supports carrying the perches. After 7 days, the traps were removed and placed individually in zip-locked plastic bags and immediately frozen at −20 °C until analysis. The mites' density in each trap was given semi-quantitatively as 0 = No mites; 1 = 1–10; 2 = 11–100; 3 = 101–1000; 4 = 1001–10,000; 5 = more than 10,000.

### Statistical analysis

All data were analysed using ANOVA (SPSS v.24 2017). Bonferroni's *post hoc* test was used to separate means only if significant main effects were detected by analysis of variance. Correlations among number and weight of droppings collected outside, vegetation score and the percentage of hens were performed.

## Results

During the study, national avian influenza alerts resulted in some periods of mandatory indoor confinement, both in autumn-winter and in spring-summer visits. For this reason, eight of the 15 flocks were withdrawn from the analysis concerning free-range use. Flocks using free-range areas show some interesting results as described below.

### Use of free-range areas

The percentage of outdoor hens in zones 1 and 2 did not show any difference; in the zone 3 there was a significant effect of farms ($p < .05$). Farms with a veranda did not show significant differences on the percentage of hens observed (Table 1).

**Table 1.** Mean percentage of hens observed in the zones considered.

| Zones | Mean | SE | *p* value |
|---|---|---|---|
| 1 | 7.190 | 0.680 | .164 |
| 2 | 5.260 | 0.760 | .096 |
| 3 | 4.180 | 1.200 | .005 |
| Veranda | 12.320 | 1.820 | .066 |
| Total | 24.030 | 2.460 | .001 |

SE: Standard error.

**Table 2.** Effects of the zone (away from the hen house) on number and weight of droppings, vegetation score and the percentage of hens observed outdoor.

| | Zone 1 | | Zone 2 | | Zone 3 | | |
|---|---|---|---|---|---|---|---|
| | Means | SE | Means | SE | Means | SE | *p* value |
| Droppings, *n* | $4.350^{a}$ | 0.550 | $2.810^{bc}$ | 0.310 | $1.800^{c}$ | 0.240 | <.001 |
| Droppings weight, g | $10.430^{a}$ | 1.270 | $6.120^{bc}$ | 0.770 | $4.190^{c}$ | 0.590 | <.001 |
| Vegetation, score | $1.520^{a}$ | 0.140 | $1.990^{a}$ | 0.190 | $2.670^{b}$ | 0.220 | <.001 |
| Total Hens Obs, % | $7.180^{a}$ | 0.680 | $5.260^{ab}$ | 0.750 | $4.180^{b}$ | 1.800 | <.006 |

Values within a row with different superscripts differ significantly.
SE: Standard error.

The percentage of hens observed outdoors in zone 3 (farther than 40 metres from the house) was affected by free-range features (i.e. free-range enrichment; $p < .05$). Indeed, farms with bushes or trees showed a higher percentage of hens in this zone. Even if no significant differences were found in the zone 1 and 2 in terms of percentage of hens observed outdoors (Table 1), as expected, zone 1 was more frequented by hens than the others.

The number and weight of droppings confirmed the higher presence of animals within 20 m from the house. The vegetation score resulted significantly better in zone 3 than in the others ($p < .001$; Table 2). Correlations among the number and weight of droppings collected outside, vegetation score and the percentage of hens showed interesting results. The percentage of hens in zone 3 was positively correlated to the vegetation score ($\rho = 0.299$; $p = .028$), while it was negatively correlated in zones 1 and 2 (n.s.).

### Parasites

Only three species of parasites were found during faecal sample analysis.

Data from faecal samples showed some differences between seasons (Table 3). *Ascaridia* eggs were higher in the spring-summer than in autumn-winter, while *Capillaria* eggs were more present in autumn-winter. The number of *Coccidia* eggs showed a significant difference between seasons ($p < .05$) (Table 3). There was significant and positive correlation between

Table 3. Effect of season on endoparasite infestation.

| | Spring–Summer | | Autumn–Winter | | |
|---|---|---|---|---|---|
| | Means | SE | Means | SE | *p* value |
| Ascaridia small | 0.440 | 0.140 | 0.770 | 0.320 | .280 |
| Ascaridia adult | 5.070 | 1.130 | 9.530 | 4.020 | .152 |
| Cestodes | 1.400 | 0.210 | 0.570 | 0.240 | .023 |
| Eggs Ascaridia Heterakis | 357.560 | 52.780 | 248.670 | 43.490 | .112 |
| Eggs Capillaria | 11.330 | 3.060 | 22.440 | 5.040 | .060 |
| Oocists Coccidia | 1825.110 | 471.740 | 647.560 | 121.320 | .016 |

SE: Standard error.

the number of *Capillaria* and *Ascaridia* eggs ($\rho = 0.232$; $p < .001$).

Regarding the presence of worms, Table 3 shows no seasonal differences in *Ascaridia*, even if the adults detected were higher in autumn-winter than in spring-summer (9.53 *vs.* 5.07). The cestodes infestation score significantly differed, being 1.4 in spring-summer and 0.57 in autumn-winter. Eight out of fifteen flocks had no red mites, and three of them showed a low infestation with approximately 10–20 red mites in the traps. Four out of 15 flocks had a moderate infestation; the mean density of red mites in the traps was 584.25 (123-919, minimum and maximum, respectively).

## Discussion

### *Use of free-range areas*

Free-range enrichment attracted more birds outside. One of the farms differed significantly in terms of the number of hens observed in all three zones, probably due to the presence of a mound covered by bushes and shaded areas. When an enriched environment was available, the percentage of outdoor hens increased in the far zone (more than 40 metres away).

In this study, hens kept in farms with a covered veranda were more attracted or less frightened to explore outside; indeed, the total number of outdoor hens was significantly higher in such farms. Even if there is a lack of studies related to the use of the veranda in free-range systems (Larsen et al. 2017), this result seems to indicate that a veranda could facilitate the hens to use the free-range area.

Gebhardt-Henrich et al. (2014) found no relationship between the percentage of hens outside the house and flock size, but individual hens in small/medium groups explored outside the house more frequently than in larger groups, indicating a flux in and out of the house in the smaller group. In the current study, smaller groups of hens were seen in the shaded areas (below bushes or trees) dust bathing, resting or showing social behaviours.

### *Parasites infestation*

This research has confirmed that helminths (*A. galli*), *Heterakis spp* and *Capillaria* are common in Italy's organic livestock, as it has been reported in other European countries (Kaufmann et al. 2011; Thapa et al. 2015; Grafl et al. 2017).

The analyses performed on intestines, however, showed that only *A. galli* and *Capillaria* were present.

In this study, a low infestation of this parasite was found, although this parasite is the most important organic farm infection in Austria, Belgium, Denmark and the United Kingdom. In other European countries (Germany, Holland and Sweden), the parasitic loads were found to be higher (Thapa et al. 2015). Thapa showed that *A. galli* has been reported throughout Europe, with an overall average prevalence of 69.5%, and that all examined hens presented approximately 10 worms each, in line with our observations. The issue of eggs, if compared to the work of Hinrichsen et al. (2016), however, makes us consider that our farms are among those with a high level of infestation. In our study, the season did not influence the helminthic load and emission of eggs, compared to a fluctuation of infections that is signalled, based on the hen's productive period (Höglund and Jansson 2011). *A. galli* eggs deposited outdoors during the winter cannot develop to infectivity in colder climates where temperatures drop below 15 °C (Tarbiat et al. 2015).

The presence of low numbers of *Capillaria* eggs suggests a mild infection. This parasite is usually reported at a lower prevalence than the other helminths (*A. galli* and *Heterakis*), as reported by Wongrak et al. (2014), while other authors have not recovered this genus from hens raised outdoors (Sherwin et al. 2013; Thapa et al. 2015). The absence of *Heterakis* spp. adults was an unexpected result, since this parasite, as already mentioned, is almost always reported in organic hens, even with variable prevalence. Thapa et al. (2015) have reported a European prevalence of 13%. The difference in infection rates in warm and cold seasons is probably due to the decreased presence of intermediate hosts in winter compared to spring/summer. The presence of coccidia is also influenced by the season, as the sporulation of the oocysts is facilitated by milder temperatures. The hens on farms checked by us, as expected, showed a low elimination of oocysts in *faeces*, as this parasitosis is typical of the first weeks of life and does not significantly affect adults. Coccidiosis mainly affects young birds; however, older flocks may experience outbreaks in association with other stressful events. Today, a reasonably safe and effective vaccine is used for almost

all pullets reared for organic egg production (and for many conventional flocks) (Berg 2001).

The low-medium infection of red mite (*Dermanyssus gallinae*) recorded by us in less than half of the flocks could be influenced by the temperature and humidity conditions that could have been unfavourable for the parasite's production of eggs. The optimum temperature to produce eggs is 25–30 °C, and the most favourable temperatures for juvenile development are between 25 and 37 °C, where developmental rates are high and mortalities are low (Maurer and Baumgärtner 1992). Their best survival rate is observed at a relative humidity (RH) of 70–90% (Nordenfors et al. 1999).

## Conclusions

In conclusion, the results showed that the presence in the free-range area of mounds, trees and bushes attracted more animals outside and allowed them to show their natural behaviour. In these conditions, even the farthest area of the range is used more often. Further evidence shows that farms with a veranda allow the hens to be more confident with their range, probably because animals find in the veranda a sort of smooth passage between the internal less illuminated environment to the bright external environment.

Regarding parasites, their presence seems to be correlated, as expected, with more intensive use of free-range areas. A low infestation by *Ascaridia galli* was found, regardless of the season. In the present study, protozoa were also found at a minimum, as this parasitosis is typical of the first weeks of life and does not significantly affect adults. Coccidiosis mainly affects young birds; however, older flocks may experience outbreaks in association with other stressful events. Because this is the first study describing the situation of free-range use and the prevalence of parasitic infestation in Italian organic farms, further studies are needed to confirm these results.

## Acknowledgements

The authors would like to thank the farmers for participating in the project.

## Disclosure statement

No potential conflicts of interest were reported by the authors.

## Ethical approval

The present work has been carried out in organic laying hens farms. None of the activities directly involved the animals for which these were agreed with the farmers.

## Funding

The project was funded by the Italian Ministry of Agriculture and Forestry and the Core Organic II Funding Bodies within the FP7 ERA-Net Project Healthy Hens (Coordination of European Transnational Research in Organic Food and Farming system, project no. 249667).

## ORCID

Susanna Lolli http://orcid.org/0000-0002-4502-4722
Guido Grilli http://orcid.org/0000-0001-6157-7258
Lorenzo Ferrari http://orcid.org/0000-0002-5177-8379
Valentina Ferrante http://orcid.org/0000-0002-8745-6777

## References

Arkle S, Guy JH, Sparagano O. 2005. Immunological effects and productivity variation of red mite *Dermanyssus gallinae* (Acari: *Dermanyssidae*). Exp Appl Acarol. 15:27–40.

Bennett DC, Yee A, Rhee YJ, Cheng KM. 2011. Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens. Poult Sci. 90:1416–1426.

Berg C. 2001. Health and welfare in organic poultry production. Acta Vet Scand Suppl. 95:37–45.

Bestman MWP, Wagenaar JP. 2003. Farm level factors associated with feather pecking in organic laying hens. Livest Prod Sci. 80:133–140.

BIOREPORT. 2016. L'agricoltura biologica in Italia. Rete Rurale Nazionale 2014–2020, pp. 29–38. Roma. 2017. ISBN 97888-8145-566-1.

Farr MM. 1956. Survival of protozoan parasite Histomonas meleagridis in faeces of infected birds. Cornell Vet. 46: 178–187.

Gebhardt-Henrich SG, Toscano MJ, Frohlich EKF. 2014. Use of outdoor ranges by laying hens in different sized flocks. Appl Anim Behav Sci. 155:74–81.

Grafl B, Polster S, Sulejmanovic T, Pürrer B, Guggenberger B, Hess M. 2017. Assessment of health and welfare of Austrian laying hens at slaughter demonstrates influence of husbandry system and season. Brit Poultry Sci. 58: 209–215.

Hegelund L, Kjaer J, Kristensen IS, Sorensen JT. 2002. Use of the outdoor area by hens in commercial organic egg production systems. Effect of climate factors and cover. In: 11th European Poultry Conference - Abst. Arch GeflüGelkd 66:141–142.

Hinrichsen LK, Labouriau R, Engberg RM, Knierim U, Sørensen JT. 2016. Helminth infection is associated with hen mortality in Danish organic egg production. Veterinary Record. 179:196.

Höglund J, Jansson DS. 2011. Infection dynamics of Ascaridia galli in non-caged laying hens. Vet Parasitol. 180:267–273.

Kaufmann F, Daş G, Sohnrey B, Gauly M. 2011. Helminth infections in laying hens kept in organic free range systems in Germany. Livest Sci. 141:182–187.

Larsen H, Cronin GM, Gebhardt-Henrich SG, Smith CL, Hemsworth PH, Rault JL. 2017. Individual ranging behaviour patterns in commercial free-range layers as observed through RFID tracking. Animals. 7:21.

Maurer V, Baumgartner J. 1992. Temperature influence on life table statistics of the chicken mite Dermanyssus gallinae (Acari: Dermanyssidae). Exp Appl Acarol. 15:27–40.

Mul M, Van Niekerk T, Chirico J, Maurer V, Kilpinen O, Sparagano O, Thind B, Zoons J, Moore D, Bell B, et al. 2009. Control methods for *Dermanyssus gallinae* in systems for laying hens: results of an international seminar. World's Poultry Sci J. 65(4):589–600.

Nagle TAD, Glatz PC. 2012. Free range hens use the range more when the outdoor environment is enriched. Asian-Australas J Anim Sci. 25:584–591.

Nordenfors H, Höglund J, Uggla A. 1999. Effects of temperature and humidity on oviposition, molting, and longevity of Dermanyssus gallinae (Acari: Dermanyssidae)). J Med Entomol. 36:68–72.

Pennycott TW, Steel F. 2001. Parasitic worms in commercial free-range poultry flocks in England and Wales. Vet Rec. 149:428.

Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J, Nansen P. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. Brit Poultry Sci. 40:439–443.

Permin A, Christensen JP, Bisgaard M. 2006. Consequences of concurrent Ascaridia galli and Escherichia coli infections in chickens. Acta Vet Scand. 47:43–54.

Sherwin CM, Nasr MAF, Gale E, Petek M, Stafford K, Turp M, Coles GC. 2013. Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. Brit Poultry Sci. 54:12–23.

SINAB 2016. Italian organics Organic numbers. http://www.sinab.it/content/bio-statistiche (Accessed 2018 May 20).

SPSS. 2017. IBM SPSS statistics for windows. Version 24.0. Armonk (NY): IBM Corp.

Tarbiat B, Jansson D, Höglund J. 2015. Environmental tolerance of free-living stages of the poultry roundworm *Ascaridia galli*. Vet Parasitol. 209:101–107.

Thapa S, Hinrichsen LK, Brenninkmeyer C, Gunnarsson S, Heerkens JLT, Verwer C, Niebuhr K, Willett A, Grilli G, Thamsborg SM, et al. 2015. Prevalence and magnitude of helminth infections in organic laying hens (*Gallus gallus domesticus*) across Europe. Vet Parasitol. 214:118–124.

Wongrak K, Daş G, Moors E, Sohnrey B, Gauly M. 2014. Establishment of gastro-intestinal helminth infections in free-range chickens: a longitudinal on farm study. Berl Munch Tierarztl. Wochenschr. 127:314–321.

Zeltner E, Hirt H. 2003. Effect of artificial structuring on the use of laying hen runs in a free-range system. Brit Poultry Sci. 44:533–537.