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

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\textbf{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.

\textbf{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.

\textbf{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\textsuperscript{2} indoors, 4 m\textsuperscript{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
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 anthelmintics 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 m²).

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).

| 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.

| Zone 1 | Zone 2 | Zone 3 |
|--------|--------|--------|
| Droppings, n | 4.350a | 2.810bc | 1.800c |
| Droppings weight, g | 10.430a | 6.120bc | 4.190c |
| Vegetation, score | 1.520a | 1.990a | 2.670b |
| Total Hens Obs, % | 7.180a | 1.800b | 4.180c |

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

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 ($9.53 \text{ vs. } 5.07$). 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; Grafi 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 parasitic load 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 hens’ 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 $15^\circ$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.

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**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.

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