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Use of diatomaceous earth as a dietary supplement in organic hens and its effects on parasite load, egg production and egg quality

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Organic farming of poultry is an established practice in the UK but parasite prevalence is not well documented even if parasitic infections may affect the flock performance. Diet supplementation with diatomaceous earth (DE) is proposed in this study in order to evaluate its efficacy for parasitic control and improved egg production and quality in layers. 400 Novogen Brown organic hens of approx. 54 weeks of age were split in two groups (*n*=200) and housed in two different barns with access to a shared field. Two diets were randomly assigned to the barns: control (basal layers diet) and intervention (basal diet with DE at 1.35%); dietary treatment lasted for 5 weeks. Faeces were sampled on a weekly basis. Faecal egg counts (FEC) were performed using the McMaster and Modified Stoll methods, and parasites were recorded by species. Egg sampling for quality tests was performed at weeks one and five of the study, collecting 15 eggs per barn for three consecutive days. Egg production was recorded on a daily basis. Parasites species identified were: nematodes *Ascaridia galli, Heterakis gallinarum, Capillaria spp*. and *Trichostrongylus tenius*; cestodes *Railletina spp*. and *Choanotaenia infundibulum*; trematodes *Echinostoma spp.* and protozoa *Eimeria spp.* Dietary treatment did not have a significant effect in parasite counts but counting method was statistically significant ($p<0.05$). Egg production was higher in the intervention group and that effect was consistent throughout the study; however, no positive effects were found with regard to eggshell quality (weight, density, egg specific gravity) or % of broken eggs. The effect of DE treatment on internal parasites was no robust, as parasite counts varied considerably between weeks. Egg production decreased as the study advanced, as a fact expected in hens of that age; still intervention group laid more eggs but these failed to prove better quality eggshells. Further studies may want to consider including DE in layers diet for a longer period (e.g. pullets until end of first year of production) or during the rearing period.

La avicultura ecológica es una práctica consolidada en el Reino Unido, sin embargo la prevalencia de parásitos internos no está bien documentada pese a que las infecciones parasitarias pueden afectar al rendimiento de la manada. En este estudio se propone la suplementación de la dieta de ponedoras con tierra de diatomeas para evaluar su eficacia para el control parasitario y mejora de la puesta y calidad del huevo. 400 gallinas ecológicas raza Novogen Brown, de aprox. 54 semanas de edad, se separaron en dos grupos (*n*= 200) y fueron alojadas en dos gallineros diferentes con acceso a un campo compartido. A cada gallinero se le

asignó una dieta al azar: control (dieta base para ponedora) e intervención (dieta base con tierra de diatomeas al 1.35%); la duración del tratamiento fue de 5 semanas. El muestreo de heces fue semanal y se realizaron recuentos parasitarios usando los métodos McMaster y Stoll modificado. Las muestras de huevos para realizar estudios de calidad se tomaron en las semanas uno y cinco, recogiendo 15 huevos por gallinero durante 3 dias consecutivos. La producción de huevos se registró a diario. Se identificaron las especies parasitarias: nemátodos *Ascaridia galli, Heterakis gallinarum, Capillaria spp*. y *Trichostrongylus tenius*; cestodos *Railletina spp*. y *Choanotaenia infundibulum*; trematódos *Echinostoma spp.* y protozoos *Eimeria spp.* El tratamiento dietético no tuvo un efecto significativo en los recuentos parasitarios pero el método de recuento resultó significativo ($p<0.05$). El grupo intervención registró una mayor producción de huevos a lo largo del estudio, sin embargo no se identificaron efectos positivos sobre la cáscara del huevo (peso, densidad o gravedad especifica) o el porcentaje de roturas. El efecto del tratamiento con tierra de diatomeas sobre parásitos internos no fue consistente, ya que los recuentos variaron considerablemente entre semanas. Conforme el estudio avanzaba, la puesta descendió según lo esperado para la edad de la manada y pese a que el grupo intervención tuvo una puesta mayor, la cascara del huevo no resultó mejor. Se podría considerar en futuros estudios la inclusion de tierra de diatomeas durante la crianza o por un periodo más largo (arranque hasta final de primer ciclo de puesta).

Palabras clave: avicultura ecológica; ponedoras; tierra de diatomeas; parásitos; huevos

Keywords: Organic farming; layers; diatomaceous earth; parasites; eggs

Introduction

Organic farming of poultry is an established practice in the UK, providing the market with a good quality and well-accepted product. Despite the robust nature of laying hens, parasitic infections are still relevant for backyard flocks, floor-reared layers and free range production systems, and they can play a role affecting the flock performance. Parasite prevalence on these systems is not well documented and significance of parasitism for production losses, disease and animal welfare implications are still to be assessed in EU countries (Van de Weerd *et al.*, 2009).

Egg size and quality of the eggshell vary as the laying period advances, resulting in a higher risk of breakage due to thinner and more fragile eggshells (Barroeta *et al.* [no date]). In addition, the detection of problems on the eggshell is the major cause of low classification, although it is argued that an intact eggshell does not exclude a poor internal egg quality (Overfield, 1996). Nutritional factors like mineral balance are key for the maintenance of good quality eggshells, therefore supplementation with bioavailable minerals involved in metabolism of eggshell is considered an strategy (Gupta, 2008)

Organic farming standards restrict the use of drugs and other synthetic chemicals in organic production, such as anticoccidials, parasiticides, antibiotics and pest control products/biocides (García Romero, 2003). Alternatives for parasite control are emerging and there are current research interests in natural products that could be used in the field to enhance poultry intestinal health and contribute to the control of pathogenic bacteria and parasites. Dietary clay supplements in the form of phyllosilicates (such as bentonite, kaolinite and talc) can have a mechanical action (binding) on pathogens and effect on feed passage, improving the absorption of nutrients (Mallet *et al*., 2005). In this context, diatomaceous earth appears as a product with potential applications in poultry farming.

Diatomaceous earth (DE) is a natural soft siliceous sedimentary rock consisting in fossilized remains of diatoms, a type of microscopic single-celled and hard-shelled algae. There are different diatom species with chemically the same silica shell (amorphous silicon dioxide) and physically an intricate perforated structure, hard and irregular. It has a variety of uses in the industry including anti-caking agent, absorbent, filtration and mild abrasive. In farming and agriculture it is used for soil improvement and plant growth, insecticide (for pest control), anti parasitic agent (wormer), and 'organic' feed additive for several farm animal species to improve body condition and final product quality.

Diet supplementation with diatomaceous earth is proposed in this study in order to evaluate its efficacy for parasitic control and improved egg production and quality.

Materials and methods

Animals and housing**:** 400 Novogen Brown hens of approx. 54 weeks of age from a commercial organic laying hen farm were split in two groups (*n*= 200) and housed in two different barns of same characteristics and size, arranged as a slat cum litter system. Each barn was equipped with roost perches, nest boxes, 10 feeders and bell drinkers. The flock had access to a shared field to free range, which was limited by an electrified fence to protect from wildlife.

Diet: two different diets were formulated and prepared in farm using a mill, and each barn was assigned a dietary treatment at random (control or intervention). Diatomaceous earth (Diature™ DE, supplied by Natural Feeds and Fertilisers Ltd.) at 1.35% was included in a diet based on home grown naked oats, wheat and peas plus protein/mineral concentrate and limestone. This supplemented diet was applied biweekly to the intervention barn and the control barn received a basal diet, formulated with the same ingredients but not including additive. Both diets were certified organic and the hens could feed *ad libitum* during the study. Dietary treatment lasted for 5 weeks.

Faecal sampling: Faeces were sampled on a weekly basis, taking three samples per barn in labeled zip bags. Floors and slat were examined and fresh faeces were collected and distributed between the bags. Bags were closed with care to eliminate all air contained inside. Samples were stored at +4ºC.

Egg sampling and production records: the sampling of eggs was performed at the beginning (week one) and at the end of the study (week five). On each of those sampling points, 15 eggs per barn were collected in colored-id cardboard trays at random for three consecutive days, making a total number of 45 eggs per treatment. Samples were stored at +2ºC. Egg production was recorded in the farm on a daily basis.

Faecal egg counts (FEC) were performed in fresh faeces within 24-48h of faecal collection, using the methods McMaster (sensitivity: 25 eggs/g faeces) and Modified Stoll (sensitivity: 5 eggs/g faeces). Both methods were performed including an initial step of mixing faeces with tap water and straining through muslin, which should help to release the parasite eggs into the water solution and to reduce faecal debris of the sample (grass, fibrous material, feathers, etc.). This was also improved by a centrifugation step in the case of the McMaster method. Two different flotation solutions were made up in the laboratory and used for the tests: salt saturated solution for the McMaster method (specific gravity, SG=1.18-1.20), and Shaether's sugar solution (SG= 1.25) for the Modified Stoll method. Only few samples were prepared simultaneously for microscopy screening and the waiting time indicated for the samples to stand in the final step was entirely respected, in order to achieve the expected level of sensitivity and accuracy of the tests. Parasite eggs were examined with the 12.5x microscope objective lens, counted and recorded separately for each parasite species. These were identified using the keys of Foreyt (2001), Kassai (1999), Kaufmann (1996), Urquhart *et al*. (1996) and Zajac and Conboy (2012).

DNA extraction and molecular biology work: total genomic DNA was extracted from all faecal samples collected along the study and freeze-dried prior extraction, using the QIAamp DNA Stool mini kit (Qiagen, Germany) with an initial bead-beating step. These were then tested by PCR using *Eimeria* species-specific primers previously described by Vrba *et al.*, (2010), to screen for all seven *Eimeria* species infecting chickens; real-time PCR was employed to quantify the presence of *E. acervulina* in faeces.

Egg quality tests: eggs were labelled and assessed individually, recording data from the following tests at the appropriate time point:

- Weight of the whole egg on digital scales.
- Candling with a homemade egg candler and external exam with natural light, identifying external defects such as shell dirtiness, hairline and star cracks, repaired fractures, pinholes, pimples, body checks and rough texture/sandpaper shells.
- Specific gravity of the egg, using nine increasingly concentrated salt solutions (SG= 1.068 to 1.098)
- Height of albumen with callipers and Haugh Units calculation.
- Weight of yolk in digital scales and weight of the albumen (calculated)
- Yolk colour with DSM Yolk Colour Fan and presence of inclusions (blood and meat spots)
- Weight of desiccated shell in digital scales, after 24h drying in a stove at 70ºC. Calculation of shell density applying the Mueller and Scott formula for surface estimation (Hughes, 1984)

Egg component analysis: total protein in yolk and albumen were determined by the application of the Dumas combustion method with the equipment vario MAX cube (Elementar GmbH, Germany).

Statistical analysis: data reported were analysed using SPSS software package version 22 (SPSS, Chicago, IL, USA). T-tests, two and one-way ANOVA were carried out. FEC data were transformed by $\log_{10}$ before statistical analysis. Significance was accepted when $p<0.05$.

Results

Parasite species identified by microscopy were:

Nematodes (roundworms): *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria spp.*, *Trichostrongylus tenius*. Cestodes (tapeworms): *Railletina spp.* and *Choanotaenia infundibulum*. Trematodes: *Echinostoma spp.* Protozoa (coccidia): *Eimeria spp.* The most commonly encountered parasite species were *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria spp.* and *Eimeria spp.* From the two previously cited parasite types, several species were present in the samples.

Dietary treatment did not have a significant effect in parasite counts ($p>0.05$). Intervention group had slightly higher overall mean counts for all parasite species (*Figure 1*)

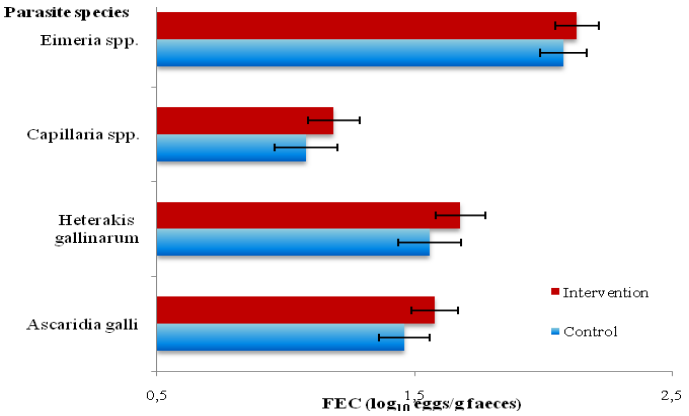

Figure 1. Average values of parasite FEC (log_{10} eggs/g faeces) by dietary treatment.

Parasite species

FEC method	**Eimeria spp.**	**Ascarids (A.galli & H.gallinarum)**	**Capillaria spp.**
McMaster	100	81.6	66.6
Modified Stoll	100	100	96.6

Table 1. FEC method sentivity. Data is expressed as % of positive replicates.

The two counting methods used had different sensitivities, which affected the estimation of parasite counts, being the overall differences by method statistically significant ($p<0.05$) (*Figure 2*). A highly significant difference ($p<0.01$) in egg counts between the two counting methods was identified for the parasite species *Ascaridia galli*, *Heterakis gallinarum* and *Eimeria spp*.; in contrast, results were not significant for *Capillaria spp.* ($p>0.05$). This fact made the two methods more or less suitable for parasite FEC depending on the type of parasites studied: Modified Stoll was a better method for nematode species whereas McMaster was more suited for coccidia (*Table 1*)

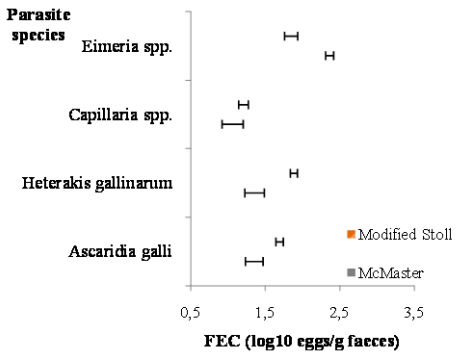

Figure 2. Average values of parasite FEC (log_{10} eggs/g faeces) by counting method.

E. acervulina was the only *Eimeria* species detected by PCR in faecal samples. Its presence was quantified by real-time PCR and results reflected a low parasite load among the flock (data not shown). Differences among treatments are yet to be assesed.

Control group produced an average of 190.9 eggs/day. Egg production was increased in the intervention group with an average of 194.9 eggs /day and that effect was consistent throughout the length of the study (*Figure 3*)

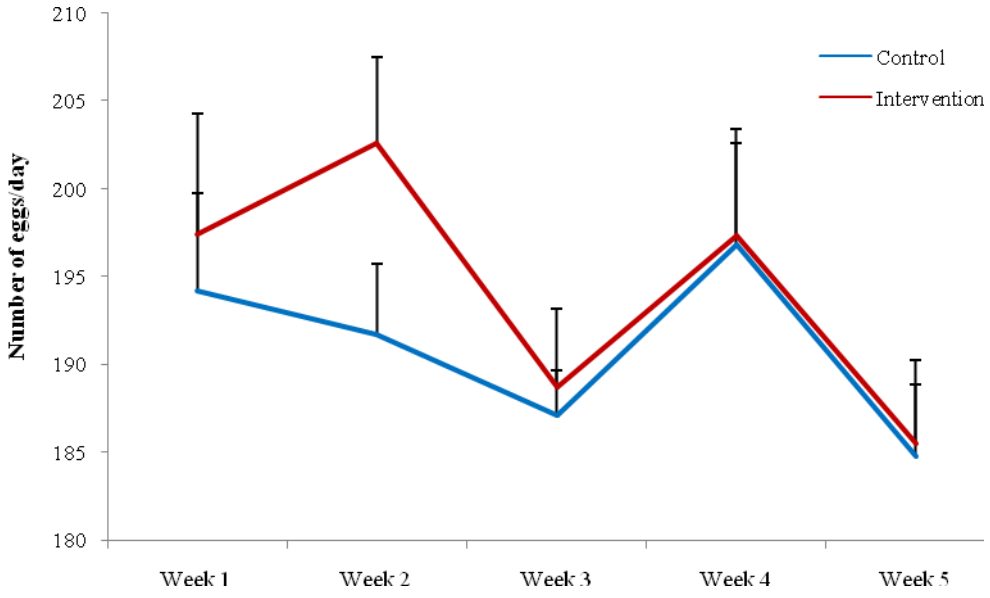

Figure 3. Average of daily egg production by dietary treatment across the time.

The proportion of dirty eggs (with blood or faeces on shell) was approximately 10% for both treatments and broken eggs (with pinhole, star or hairline cracks) accounted 6.7% in control group and 4.4% in intervention group, with no change between week 1 and 5.

Weights of both yolk and albumen increased slightly for both treatments (only control yolk weight decreased slightly). Average weights were approximately 18.5g for yolk and 41g for albumen. Both treatments increased the values of Haugh Units, having similar results by week 5 (around 75 HU)

An increase of approximately 1.5 points in the mean values of yolk colour, measured with the DSM Yolk Colour Fan scale, was identified for both treatments. Up to one third of the eggs examined presented inclusions (blood or meat spots); the number of meat spots decreased for both treatments but blood spots increased in the intervention group.

The average egg weight increased at the end of the study for the control group and stayed stable for the intervention group (mean value around 66g/egg). Eggshells were slightly lighter for the intervention group at week 5 compared to week 1, while values of control group almost did not change.

Eggshell density is defined as: weight of dried eggshell / surface of the shell, being surface of the shell the result of the calculation 4.67 x (weight of whole egg)$^{2/3}$ (Mueller and Scott formula). Intervention group decreased in eggshell density by a mean of 4mg/cm^2, and control group stayed stable. A similar situation was observed in the specific gravity (SG) values: intervention group had higher SG values in week 1 but decreased SG by 4 points at the end of the study; control group accounted an stable SG value of 1087.

Discussion

Diverse types of parasite species were identified in the present study.

Variation in parasite counts for the different species was found between weeks. This might be expected even if established natural immunity to parasites would be assumed in hens of that age, as other factors like differences in parasite life cycles intervene, and fluctuations in the hen's immunity can occur (Lunden *et al*., 2000).

Residual contamination by parasite eggs and the choice of disinfection procedures in production sites have been considered risk factors for coccidia and helminth parasitic infections (Lunden *et al*., 2000; Höglund and Jansson, 2011). In addition, free range poultry production systems have a higher risk of other parasitic infections, due to the access to pastures (Permin *et al.*, 1999). These can be potentially infected with parasites and support intermediate hosts (like earthworms, slugs, snails and arthropods), which are essential for the transmission of parasites with indirect life cycle. In addition, ranging birds generally have a greater bird to bird contact and exposure to faecal material, which increases the chances of infection. Nevertheless, by having access to the outdoors, the pressure of contamination and infection inside the housing sheds is substantially reduced (Pont, 2009)

Dietary treatment did not have a significant effect in parasite counts, and these results do not contradict the findings of Bennet *et al.* (2011), who concluded that the effect of DE on internal parasites of hens was no robust. Although several *Eimeria* species were identified by microscopy, the fact that the only one detected by PCR and quantified by qPCR was *E. acervulina* could be explained by the fact that the limit of sensitivity of the test is set at 10 copies of the target sequence, but in practice detection is also influenced by the DNA extraction and the presence of contaminants or PCR inhibitors in the faecal sample (Vrba *et al*., 2010)

Egg production was increased in the intervention group and that effect was consistent throughout the length of the study. Even so, the number of eggs laid by the flock decreased as the study advanced, as a fact expected in hens getting through their last third of first year of production, although numbers suffered some fluctuation between weeks. That is to say that the weather was not good between weeks 2 and 4, with storms, increased rainfall, strong winds and lower temperatures. This could have affected negatively both egg production and parasite counts (increased numbers, data not shown).

Considering the flock age and timepoint in egg production, it was expected to see an increase on egg weight, which was more marked in control group, and a decrease on eggshell densities, only seen in intervention group. No positive effects with regard to eggshell quality (weight, density, egg specific gravity) or % of broken eggs were identified for the group supplemented with DE. This contrasts with the results of Bennet *et al*. (2011), who reported significantly higher egg production in hens fed DE for both different breeds, Llohmann Brown and Bovan Brown; the latter breed also showed an improvement in egg quality (larger eggs and heavier, thicker shells) under DE dietary treatment.

In conclusion, further studies should be carried out to determine the efficacy of diatomaceous earth for parasitic control, improved egg production and quality. These may want to consider including DE in the diet of hens for a longer period, e.g. from pullet age until end of first year of production, or during the rearing period to evaluate parasitic infection dynamics.

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