

# Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens

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**ABSTRACT** The effectiveness of diatomaceous earth (DE) as a treatment against parasites and to increase feed efficiency and egg production of organically raised free-range layer hens was evaluated in 2 breeds of commercial egg layers [Bovan Brown (**BB**) and Lowmann Brown (**LB**)] that differ in their resistance to internal parasitic infections. Half the hens of each breed were fed diets supplemented with DE (2%). Their internal parasite loads were assessed by biweekly fecal egg counts (**FEC**) and by postmortem examination of the gastrointestinal tract. Supplementing DE in diets of LB hens, the more parasite-resistant breed, did not significantly affect their FEC and adult parasite load. However, BB hens treated with dietary DE had significantly lower *Capillaria* FEC, slightly lower *Eimeria* FEC, fewer birds infected with *Heterakis*, and significantly lower

*Heterakis* worm burden than control BB hens. Both BB and LB hens fed the diet containing DE were significantly heavier, laid more eggs, and consumed more feed than hens fed the control diet, but feed efficiency did not differ between the 2 dietary treatments. Additionally, BB hens consuming the DE diet laid larger eggs containing more albumen and yolk than hens consuming the control diet. In a subsequent experiment, the effectiveness of DE to treat a Northern fowl mite (*Ornithonyssus sylviarum*) infestation was tested. Relative to controls, both breeds of hens that were dusted with DE had reduced number of mites. The results of this study indicate the DE has the potential to be an effective treatment to help control parasites and improve production of organically raised, free-range layer hens.

**Key words:** organic layer production, diatomaceous earth, parasite control, egg quality, egg production

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## INTRODUCTION

Consumers are increasingly concerned with the safe and ethical production of their food. The demand for organically produced animal products, including organic poultry eggs, has been steadily increasing (Berg, 2001; Patterson et al., 2001; Kouba, 2003; Oberholtzer et al., 2006; Bejaei and Cheng, 2010). This has led to an increase in the production of both free-range and organic poultry in many countries. In organic poultry production, standards ban the use of synthetic chemicals and require birds to have access to the outdoors. However, free-range production of poultry has a higher risk of parasitic infections (Permin et al., 1999). In organic

farming, the routine uses of prophylactic medications are not allowed. 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 anemia (Reid and Carmon, 1958; Ruff, 1999; Permin et al., 2006). Therefore, an effective and safe method is needed for the treatment of parasites in organic animal production.

One proposed treatment to control external and internal parasites is to add diatomaceous earth (**DE**; Macy, 2000) to the diet of production animals (Canadian Organic Growers, 2000). Diatomaceous earth consists of fossilized diatoms and is made up of almost pure amorphous silicon dioxide. Diatomaceous earth has been recognized as an effective insecticide. It works mainly by absorbing the waxy outer cuticle of insects upon contact, causing death by desiccation (Quarles, 1992; Fields, 2000). To a lesser extent, the abrasive property of DE also aids in the damage of the cuticle (Quarles, 1992; Korunic, 1998). It is commonly used as a protectant against invertebrate pests in grain storage. Diatomaceous earth has been shown to decrease the

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number of ectoparasites in Tree Swallow (*Tachycineta bicolor*) nests (Dawson, 2004) and to reduce poultry red mite (*Dermanyssus gallinae*) survival in vitro (Maurer et al., 2009). Diatomaceous earth with less than 7% composition of crystalline silica is generally recognized as a safe food additive in Canada and the United States (Fields, 2000). For controlling internal parasites, DE is often promoted by testimonies and product claims to be effective and safe for livestock, but little scientific research has been performed to judge its efficacy. It has been suggested that DE may provide trace minerals that help the host cope with parasite burdens (McLean et al., 2005). The use of DE to control internal parasites in ruminants has been tested with mixed results (Fernandez et al., 1998; McLean et al., 2005) and no research has been performed to evaluate its efficacy in poultry.

It has also been claimed that feeding DE to laying hens can increase feed efficiency and egg production (Eshleman, 1966). Mathis and McDougald (1995) found that feeding DE significantly improved feed conversion in broilers. In the present study, we evaluated the effectiveness of DE as a supplement against external and internal parasites of organically raised free-range layer hens and at the same time evaluated its effects on egg production and egg quality. Two commercial breeds of brown-egg-laying hens, Bovan Brown (**BB**) and Lohmann Brown (**LB**), were used for the experiment. Previous studies have shown that LB hens are genetically more parasite resistant than other breeds (Permin and Ranvig, 2001; Gauly et al., 2002, 2008).

## MATERIALS AND METHODS

This study was conducted at the Centre for Sustainable Food Systems of the University of British Columbia (**UBC**; Vancouver, British Columbia, Canada) and was conducted during the summer and fall months. Hens were maintained in accordance with the guidelines of the Canadian Council of Animal Care, and all procedures were approved by the UBC Animal Care Committee (certificate no. A08-0110).

**Table 1.** Nutrient composition of layer diet (as fed basis)<sup>1</sup>

Item	Control <sup>2</sup>	DE <sup>3</sup>
Moisture (% as fed)	11.8 ± 0.3	10.2 ± 0.6
Protein (% DM)	18.1 ± 0.3	18.9 ± 0.6
Fat (% DM)	6.1 ± 0.8	5.3 ± 0.3
Carbohydrates (% DM)	62.3 ± 2.5	59.6 ± 2.2
Ash (% DM)	13.7 ± 1.5	16.1 ± 1.9
Gross energy (kcal/kg)	3,757 ± 18	3,617 ± 94

<sup>1</sup>Proximate analysis of diet determined according to AOAC (2000).

<sup>2</sup>All-purpose certified organic poultry grower mash supplied by In-Season Farms Inc. (Abbotsford, British Columbia, Canada).

<sup>3</sup>Layer diet supplied with 2% diatomaceous earth (DE). Source of DE was Red Lake Earth (Absorbent Products, Kamloops, British Columbia, Canada), which contains 65% DE and 35% montmorillonite.

## Source of DE

The source of DE used in this study was Red Lake Earth (Absorbent Products, Kamloops, British Columbia, Canada), which contains 65% DE and 35% montmorillonite. This product was chosen because it is approved for use in animal feeds in both Canada (CFIA registration no. 999094) and the United States (FDA registration no. 10370895308) and is listed by the Organic Materials Review Institute (Eugene, OR).

## Experimental Birds

A total of 57 BB and 62 LB hens, both commercial brown egg layers, were used in this study. These were obtained as 1-d-old chicks from local commercial hatcheries. Chicks were raised in floor pens at the UBC farm and fed ad libitum with an all-purpose certified organic poultry grower mash (In-Season Farms Inc., Abbotsford, British Columbia, Canada). Chicks were beak trimmed shortly after hatching and vaccinated for Newcastle and bronchitis at 2 wk of age.

At 11 wk of age (first week of May 2008), all birds were wing-banded and divided into 4 treatment groups: 29 BB and 31 LB were placed in the experimental groups, and 28 BB and 32 LB remained in the control groups. The experimental groups were fed diets supplemented with 2% DE. All 4 groups were transferred to hen houses that provided at least 0.2 m<sup>2</sup> of floor space and 0.25 m of roost space/bird. The pasture was sectioned by an electric fence. Birds were given daily access to pasture, with about 7.5 m<sup>2</sup>/bird, from approximately 0830 to 2100 h. Feed was provided only in the pasture but was refilled every morning and emptied at night to discourage rodents. Water was provided ad libitum inside the hen house via a nipple drinker system and outside via a bell waterer. At 18 wk of age, all hens were switched from the grower mash to an organic layer mash (In-Season Farms Inc.; Table 1), and the experimental groups continued to receive DE supplements in the diet. The hens were also given access to dust baths and shelters in the pasture. The groups receiving DE in the diet were also provided with DE mixed in their dust baths (20%) and in the shavings in the nest boxes (10%).

## Parasite Study

### Fecal Egg Counts and External Examination.

Forty hens (10 hens/diet per breed) were randomly selected and repeatedly examined at biweekly intervals between 16 and 28 wk of age (June–September 2008) and again at time of killing (33–38 wk of age; October–November 2008). Fecal collection was performed by placing hens into raised, individual wire cages with wax paper below before feeding in the morning. Typically, hens excreted within 10 to 20 min of being placed

into the cage. Approximately 4 g was collected into preweighed 50-mL centrifuge tubes. Hens were then visually examined for any signs of external parasites before being returned to their enclosures. Particular attention was paid to the legs, comb, wattles, inside the mouth, under the wings, and under the vent area. Fecal samples were transported to the laboratory, where they were weighed, preserved in 10% formalin using a 1:1 ratio of volume to fecal mass, and refrigerated at 4°C until being examined (within 1 wk).

Parasite eggs were quantified using a modified Wisconsin sugar flotation method (Cox and Todd, 1962; Cox and Lemiski, 1989). The formalized samples were diluted with distilled water to 35 mL, vortexed, and centrifuged at  $500 \times g$  for 7 min. Most of the supernatant was then decanted, and Sheather's solution (specific gravity = 1.2) was added at a volume 11.5 times the mass of excreta. This mixture was then homogenized with a glass rod, vortexed, and the contents were inverted several times immediately before loading into both chambers of a McMaster slide. The slide was left to stand 2 to 3 min before viewing under a light microscope. Parasite eggs were identified according to Soulsby (1982), Foreyt (2001), and Zajac and Conboy (2006).

Because no external parasites were detected in all the hens in 2008, a supplemental study was conducted in 2009 when heavy infestation of northern fowl mites occurred. A total of 26 BB and 35 LB hens in their second laying cycle (82 wk of age) were used. All hens were naturally infested with northern fowl mites (*Ornithonyssus sylviarum*) at the start of the experiment. Hens were housed as described in the 2008 study (see previous) and were fed a certified organic poultry layer mash (In-Season Farms Inc.) that was not supplemented with DE.

Each hen was individually weighed and scored for mites (see below) independently by 2 investigators (DCB and YJR). Hens were then divided into 2 experimental groups, each containing equal numbers of both breeds, and placed into adjacent pens. After 4 d, to allow hens to adjust to new pen mates, hens were individually dusted with either fine sand (control group) or DE (experimental group) and returned to the appropriate pen. Hens were dusted by placing them into a bin and rubbing either sand or DE into feathers on the thighs, abdomen, back, and vent area. The roosts, nest boxes, and floor litter were also dusted with either fine sand (control group) or DE (experimental group). One week later, hens were individually weighed, scored for mites, and dusted again. The roosts, nest boxes, and floor litter were also dusted. Individual hens were again weighed and scored for mites 1 wk later.

Hens were scored for degree of mite infestation using 2 methods. First, a subjective visual score (0–5) based on the presence and concentration of mites was independently assigned by the same 2 investigators. Second, a piece of tape (approximately 3.5 cm  $\times$  5 cm) was used

to collect mites from the base of the thigh feathers on one side of each hen. Taped mites were then examined and counted under a dissecting microscope. Many of the birds were heavily infested and emaciated; it was felt that subjecting the birds to further stress was unwise. Hence, blood samples were not obtained for hematological and immune parameters.

**Phytohemagglutinin Skin Test.** At 31 to 32 wk of age, the immune status (T cell-mediated immunity) of all 40 focal hens was assessed by the phytohemagglutinin (PHA) skin test (Cheng and Lamont, 1988; Kean and Lamont, 1994; Smits et al., 1999). Briefly, a small patch of skin on the web (patagia) of both wings was plucked and marked. The hens were injected with 50  $\mu$ L of PHA (L8754, Sigma-Aldrich, St. Louis, MO) in 50  $\mu$ L of Dulbecco's PBS (D8537, Sigma-Aldrich, St. Louis, MO) into the marked site of one wing. The marked site of the other wing was injected with the same volume (50  $\mu$ L) of Dulbecco's PBS to control for nonspecific inflammation. The thickness of the wing web of each marked site was measured 3 times immediately before injection and again 24 h after injection with microcalipers (accuracy 0.01 mm). Wing web swelling was calculated as the difference between the thickness of the wing web before and after (24 h) injection. The cell-mediated immune response (wing web index) was calculated as the difference in wing web swelling between the PHA-injected and saline-control sites.

**Postmortem Examination.** Between 33 and 38 wk of age, all 40 of the focal hens (10 from each group) that were closely followed with FEC were killed and a postmortem examination was performed to assess the adult parasite load. After an overnight fast, 3 to 4 individual hens per day were randomly selected and placed into cages for collection of their excreta. They were then transported to the laboratory, weighed, and killed by decapitation. Trunk blood was collected into heparinized vacutainers (Becton Dickinson and Co., Franklin Lakes, NJ). The gastrointestinal tract was exposed and segmented into the esophagus, crop, proventriculus, ventriculus, small intestine, cecum, and rectum. Incisions were made to expose the contents, and any visible parasites were extracted with forceps. Contents in the organs were flushed under tap water over a 60 mesh sieve with 250- $\mu$ m aperture. Retentate was then backwashed into a Petri dish and further examined for more worms under a stereo microscope. All extracted parasites were preserved in 70% ethanol. Adult parasites were viewed under stereo and light microscopes and identified according to Dunn (1969) and Grist (2004).

Blood smears were made from the trunk blood collected. Smears were allowed to dry and then stained using a Hemacolor staining kit (EMD Chemicals Inc., Gibbstown, NJ). Differential white cell counts were conducted using 1,000 $\times$  microscopy. For each blood smear, 100 white cells were counted and the heterophil:lymphocyte (H:L) ratio was determined.

## Production

Between 16 and 32 wk of age, BW of individual hens and pen feed intake were measured at 4-wk intervals. Body weight was measured in the morning before the hens were released into the outdoor pens, and hence before the hens were fed. Daily egg production per pen was recorded for 5 d/wk. Once every 4 wk, between 20 and 32 wk of age, all the eggs laid on 1 d were collected, weighed, and stored overnight at 4°C. Eggs were broken out onto a level glass surface and the height of the albumen was measured using a standard tripod micrometer. The yolk was weighed and its color was measured with a Roche yolk color fan scale. Shells were washed, dried, and weighed. The albumen mass was then calculated by difference.

Between 33 and 38 wk of age, 10 hens from each group were killed and a postmortem examination was performed to assess the adult parasite load (see previous section) and bone mineralization. The BW, egg production, and egg quality were again measured at 38 wk of age.

## Statistical Analyses

Data are reported as means  $\pm$  SE and were analyzed using JMP statistical software (version 7, SAS Institute Inc., Cary, NC). Individual FEC and worm counts were transformed by  $\log_{10}(\text{count} + 1)$  transformation before statistical analysis to normalize the distribution. The BW of individual hens, transformed FEC, and mite scores were analyzed by 2-way ANOVA with repeated measures. Differences between treatments and breeds in the number of hens infected with worms and in the transformed worm counts were assessed by Fisher's exact test. Wing web index, H:L ratio, egg production, and egg size were analyzed by ANOVA. Relationships among parameters were assessed by simple correlation. Significance was accepted when  $P < 0.05$ .

## RESULTS

### Effect of Dietary DE on Internal Parasites

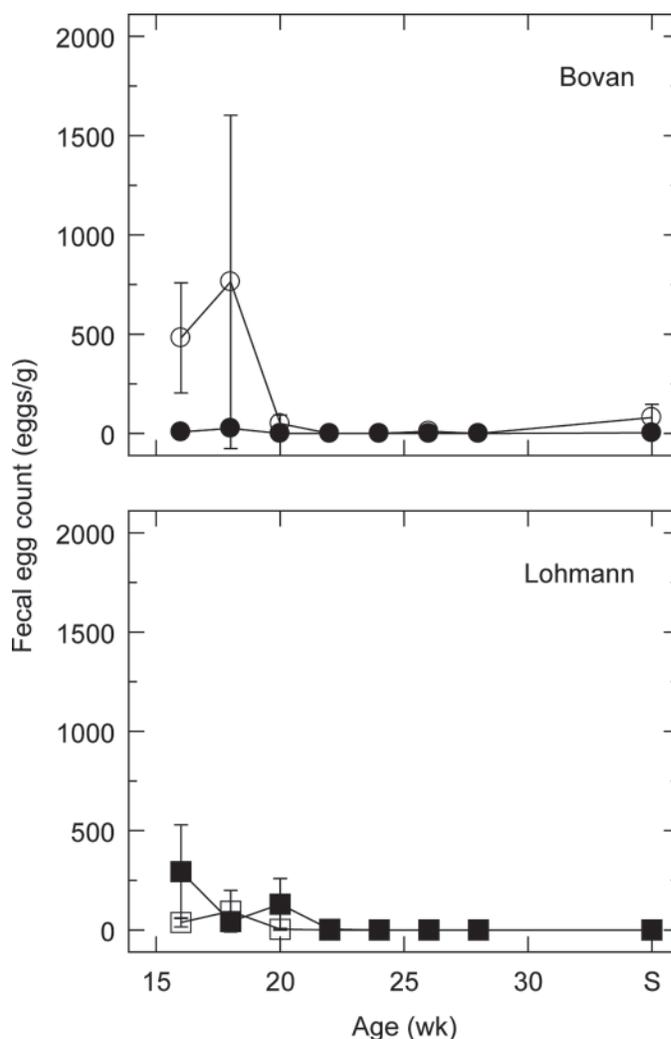
**FEC.** Eggs from 1 protozoan, *Eimeria* spp., and 3 helminths, *Ascaridia* spp., *Heterakis* spp., and *Capillaria* spp., were identified in the FEC. *Eimeria* FEC were highest at the start of the experiment and subsequently declined by the second fecal exam ( $P = 0.00004$ ; Figure 1). Control BB hens tended to have higher *Eimeria* FEC than DE-treated BB hens, but DE did not affect *Eimeria* FEC in LB hens (breed  $\times$  diet interaction,  $P = 0.07$ ).

Control BB hens had significantly ( $P = 0.003$ ) higher *Capillaria* egg counts than DE-treated BB hens (Figure 2). However, the same difference was not observed with LB hens. As the season progressed, *Ascaridia* egg counts increased significantly ( $P = 0.02$ ) in BB hens regardless of dietary treatment, but no significant increase was

observed with the LB hens (Figure 3). *Ascaridia* egg counts in LB hens remained low throughout the season. The FEC of *Heterakis* remained low throughout the season and did not differ between LB and BB, nor were they affected by dietary treatments.

**Postmortem Examination.** Postmortem examinations are summarized in Table 2. Only adult *Ascaridia* spp. and *Heterakis* spp. nematodes were recovered. Although *Capillaria* spp. eggs were detected in some of the excreta collected on the day of the postmortem examinations (Figure 2), no adult *Capillaria* worms were recovered (likely small enough to pass through the sieve screen). *Ascaridia* was found predominantly in the upper portion of the intestine and on few occasions in the rectum, and once in the cecum of a bird. *Heterakis* was found only in the cecum.

The number of hens infected with *Ascaridia* and *Heterakis* did not differ between breeds, nor was it affected



**Figure 1.** Effect of dietary diatomaceous earth on fecal egg counts of *Eimeria* spp. in free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Hens were killed (S) between 33 and 38 wk of age.

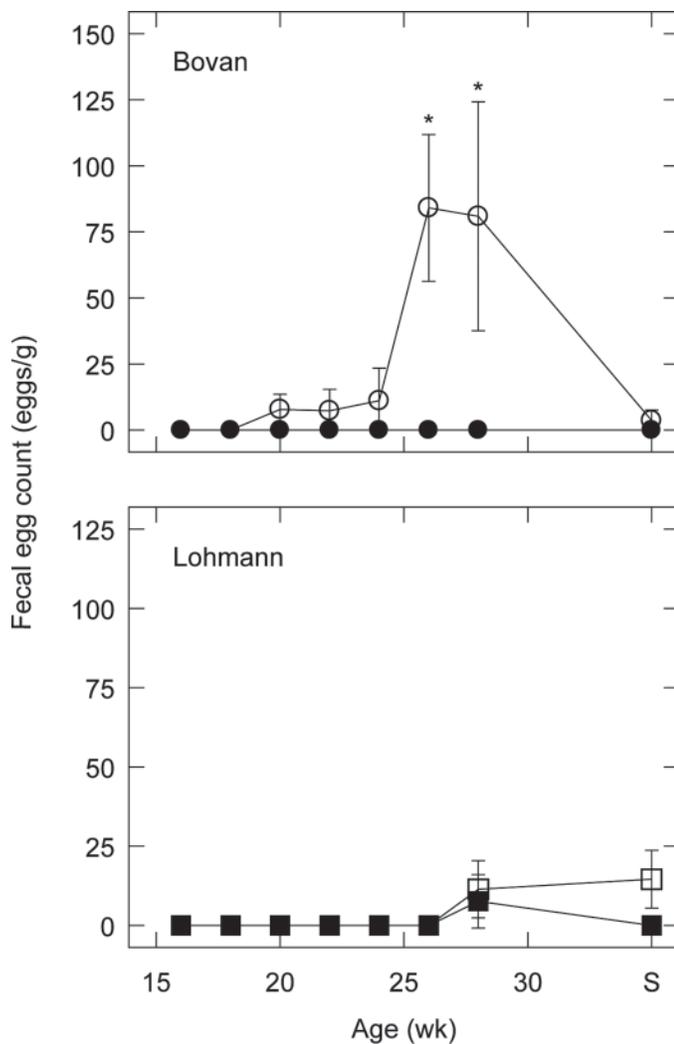
**Table 2.** Comparison of adult nematodes recovered after postmortem examinations between hens supplemented with diatomaceous earth (DE) and those on the control diet<sup>1</sup>

Item	Bovan		Lohmann	
	Control	DE	Control	DE
<i>Ascaridia</i> spp.				
Birds infected <sup>2</sup> (%)	70	70	30	50
Worm burden <sup>3</sup>	14.6 ± 9.0	17.5 ± 4.8	5.0 ± 3.5	2.6 ± 1.0
<i>Heterakis</i> spp.				
Birds infected <sup>2</sup> (%)	70	30	60	70
Worm burden <sup>3</sup>	20.2 ± 10.5	1.7 ± 0.7	3.3 ± 0.8	10.4 ± 2.6

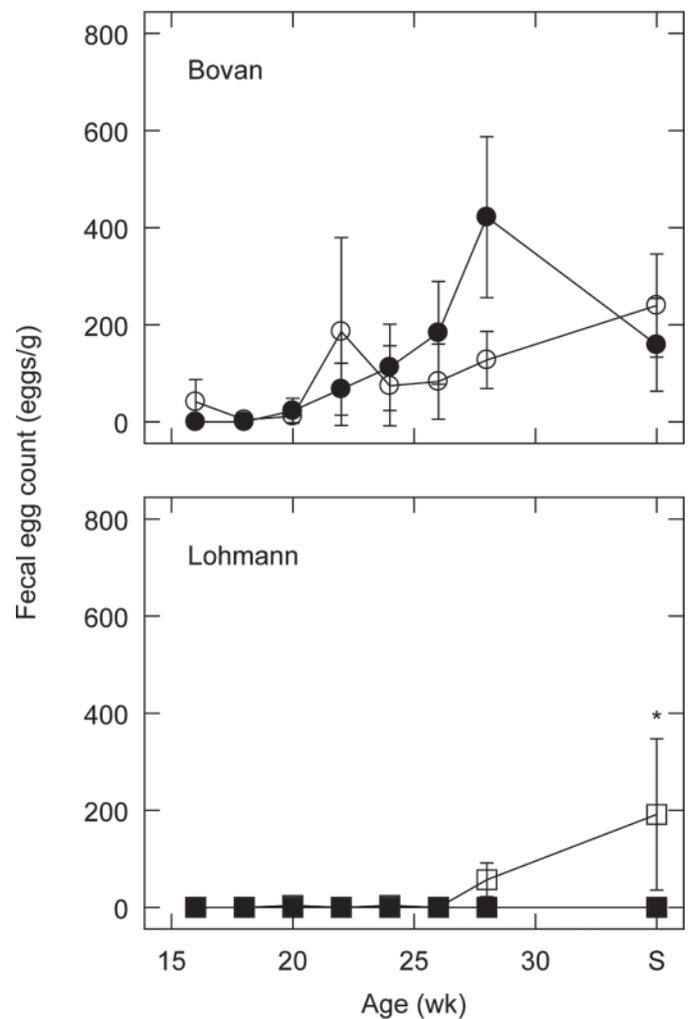
<sup>1</sup>Two breeds of chickens were compared (Bovan Brown and Lohmann Brown), with 10 hens tested under each treatment and breed.

<sup>2</sup>Determined on a basis of whether the parasites were present or absent.

<sup>3</sup>Mean number of worms per infected hen.



**Figure 2.** Effect of dietary diatomaceous earth on fecal egg counts of *Capillaria* spp. in free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Hens were killed (S) between 33 and 38 wk of age. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).



**Figure 3.** Effect of dietary diatomaceous earth on fecal egg counts of *Ascaridia* spp. in free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Hens were killed (S) between 33 and 38 wk of age. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).

**Table 3.** Comparison of phytohemagglutinin (PHA) skin test and heterophils:lymphocyte (H:L) ratio between hens supplemented with diatomaceous earth (DE) and those on the control diet<sup>1</sup>

Item	Bovan		Lohmann	
	Control	DE	Control	DE
PHA	0.070 ± 0.012	0.039 ± 0.009	0.062 ± 0.014	0.068 ± 0.010
H:L ratio	0.31 ± 0.05	0.37 ± 0.04	0.34 ± 0.04	0.36 ± 0.04

<sup>1</sup>Two breeds of chickens were compared (Bovan Brown and Lohmann Brown), with 10 hens tested under each treatment and breed.

by DE treatment. When comparing the worm burden, BB appeared to be more susceptible to *Ascaridia* infections than LB given that significantly ( $P < 0.05$ ) more BB were infected and tended ( $P = 0.10$ ) to have a higher mean worm burden than LB.

**PHA Skin Test and H:L Ratio.** Wing web index and H:L ratio did not differ between breeds nor between treatments (Table 3) and were not correlated ( $r = -0.21$ ;  $P = 0.15$ ). Both parameters were also unrelated to FEC and worm burden (data not shown).

### Effect of Externally Applied DE on Northern Fowl Mites

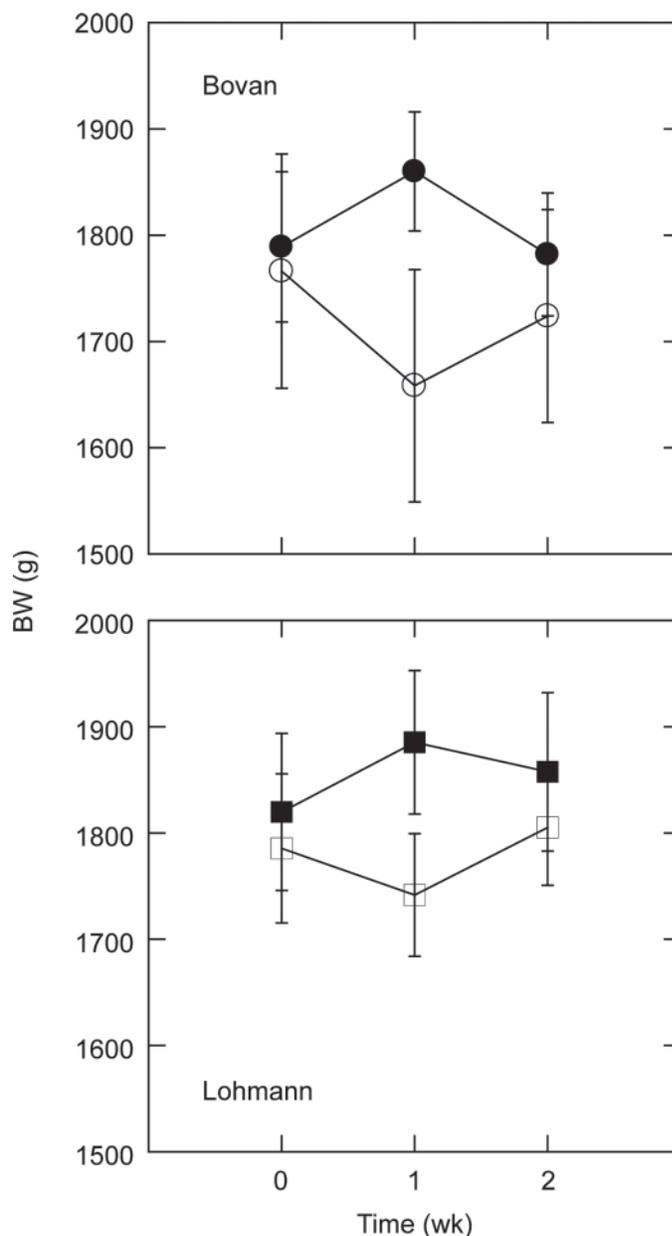
Body weight (Figure 4) and mite scores (Figure 5) did not differ significantly between breeds or treatments at the start of the experiment. After dusting with DE, hens of both breeds were significantly ( $P = 0.02$ ) heavier and had significantly ( $P = 0.005$ ) fewer mites compared with control hens that were dusted with sand. The DE was equally effective in reducing mite counts on both BB and LB hens. Significant interactions between dietary treatment and age were found for BW, egg production, and egg quality parameters.

### Effect of DE on BW and Egg Production

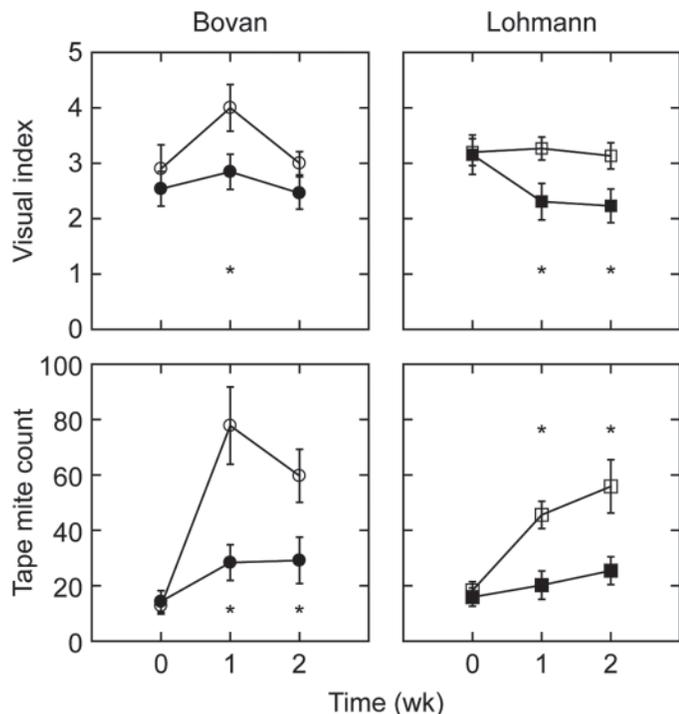
**Pre-lay Period.** Regardless of dietary treatment and breed, BW (Figure 6) and egg production (Figure 7) increased sharply between 16 and 20 wk of age, with hens reaching 50% production between 18 and 19 wk of age. These parameters exceeded the recommended management targets (Centurion Poultry, 2008; Lohmann Tierzucht GmbH, 2008).

**Laying Period.** Both BB and LB hens fed the diet containing DE continued to increase BW and egg production up to 32 wk of age. However, control hens initially lost weight and had reduced egg production. As a result, hens fed the diet containing DE were significantly heavier (Figure 6) and laid more eggs (Figure 7) than hens fed the control diet. In addition to higher egg production, BB hens consuming the DE diet also laid larger eggs containing more albumen and yolk than BB hens consuming the control diet (Figure 8). Albumen height and yolk color were unaffected. The DE did not affect egg size or egg quality of LB hens.

The BW of control hens began increasing after 28 wk of age, such that by 38 wk of age the differences attrib-



**Figure 4.** Effect of externally applied diatomaceous earth on BW of free-range organic laying hens infested with northern fowl mites. Two breeds of commercial brown-egg-laying hens, Bovans (circles) and Lohmanns (squares), were fed a certified organic layer mash. Hens were dusted with either sand (open symbols) or diatomaceous earth (filled symbols).



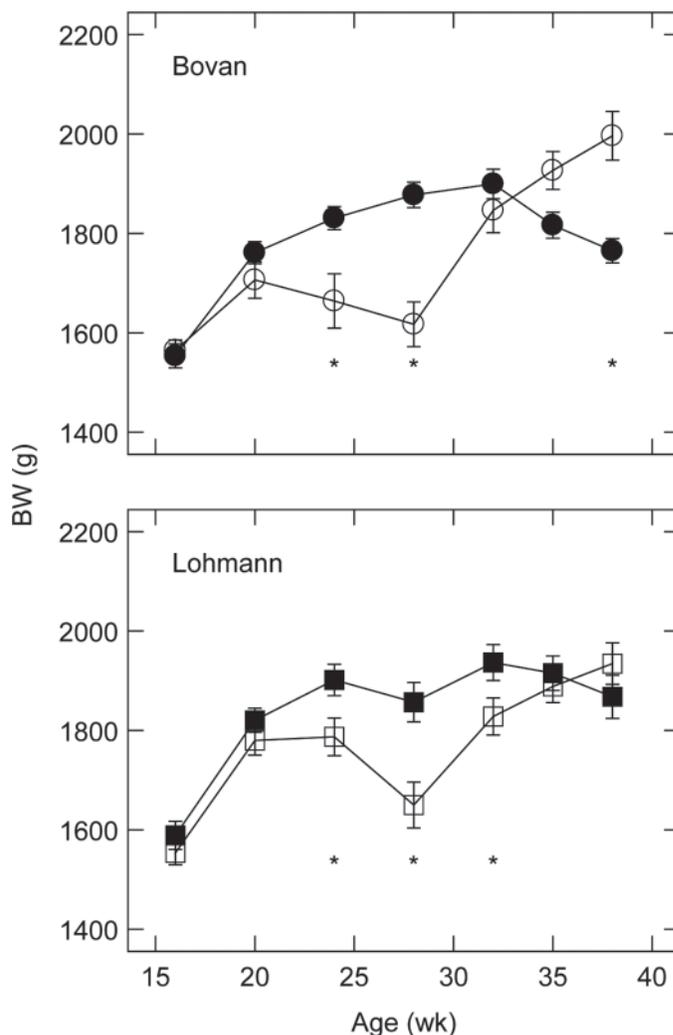
**Figure 5.** Effect of externally applied diatomaceous earth on mean mite scores (visual index, tape mite count) of free-range organic laying hens infested with northern fowl mites. Two breeds of commercial brown-egg-laying hens, Bovans Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash. Hens were dusted with either sand (open symbols) or diatomaceous earth (filled symbols). Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).

utable to dietary DE had either disappeared (LB hens) or were reversed (BB hens). Between 20 and 28 wk of age, egg shell weight and thickness were significantly greater in both BB and LB hens fed the diet containing DE (Figure 9). However, after 28 wk of age, egg shell quality improved for hens fed the control diet whereas it declined for hens fed DE. By 38 wk of age, egg shell weight and thickness were significantly greater in hens fed the control diet.

The average feed intake of control hens was 137 g/hen per day (Figure 10), which is within the range of that expected for free-range hens (Bubier and Bradshaw, 1998; Hegelund et al., 2006; Horsted et al., 2007). Hens fed the DE diet consumed significantly ( $P < 0.006$ ) more feed than those fed the control diet (Figure 10), averaging 145 g/hen per day. Feed efficiency (g of feed/g of egg) did not differ between the 2 dietary treatments (Figure 10).

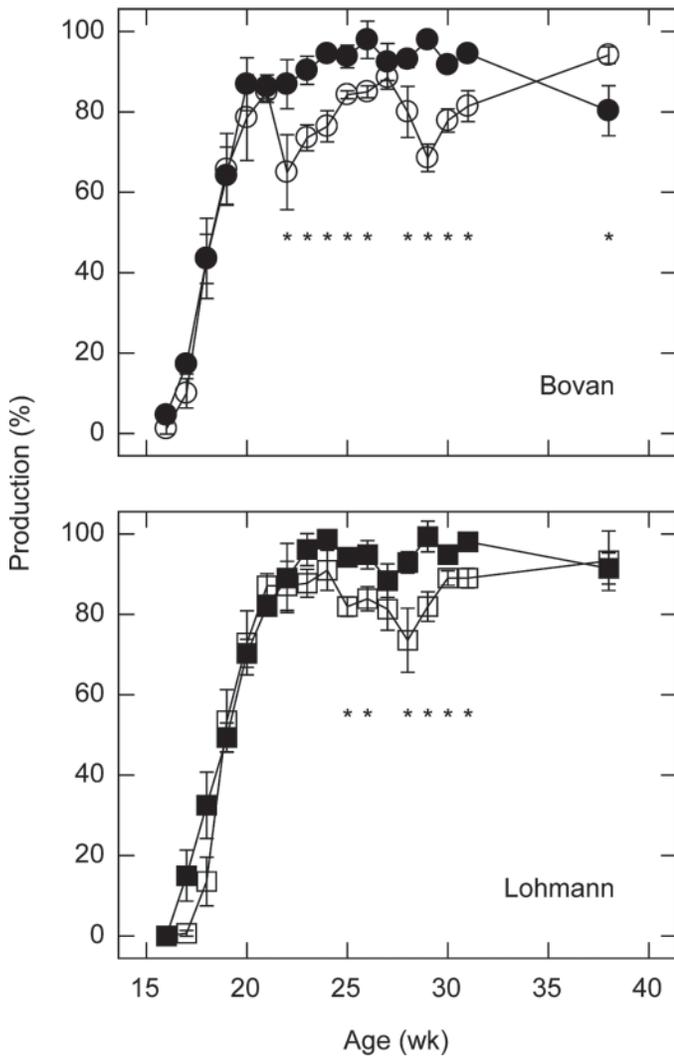
## DISCUSSION

This study tested the claim that feeding DE to laying hens can increase resistance to internal parasites, feed efficiency, and egg production. Two breeds of commercial hens (BB and LB), maintained under a free-range organic management system, were fed a diet containing 2% Red Lake Earth (product contains 65% DE and 35% montmorillonite) during the egg laying season.



**Figure 6.** Effect of dietary diatomaceous earth on BW of free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovans Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).

Compared with control BB hens, control LB hens had lower FEC for most of the internal parasites detected as well as fewer birds infected and a lighter worm burden in postmortem examination. This is consistent with previous observations that LB hens are genetically more parasite resistant than several other commercial and indigenous breeds examined (Permin and Ranvig, 2001; Gauly et al., 2002, 2008). Supplementing 2% DE in diets of LB hens did not significantly affect their FEC and adult parasite load. On the other hand, BB hens treated with dietary DE had significantly lower *Capillaria* FEC, slightly lower *Eimeria* FEC, fewer birds infected by *Heterakis*, and a significantly lower *Heterakis* worm burden than control BB hens. Each individual parameter may not be strong, but together they provide convincing evidence. We therefore conclude that the effect of DE on internal parasites was not robust. It did not improve resistance in birds that were genetically more resistant but may help birds



**Figure 7.** Effect of dietary diatomaceous earth on egg production of free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).

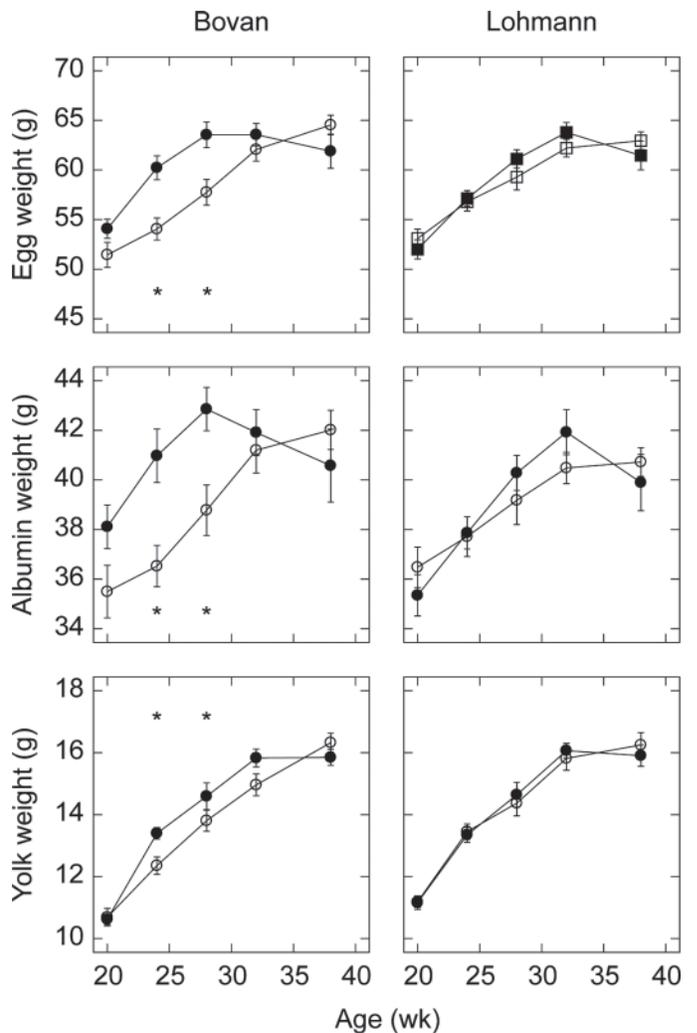
that were less resistant to lower their parasite load. We have also confirmed that DE is effective in controlling northern fowl mites in both BB and LB when applied externally.

In this study the H:L ratio was used to assess long-term stress (Gross and Siegel, 1983) in the hens. This ratio did not differ between the 2 breeds or between the controls and DE treatment (Table 3) and was unrelated to the parasite burdens of the hens. The relatively low H:L ratio observed in this study probably is indicative that the hens were not unduly stressed (Gross and Siegel, 1983; Singh et al., 2009). However, because this ratio was assessed only at the end of the experiment, habituation or adaptation to chronic stress cannot be ruled out.

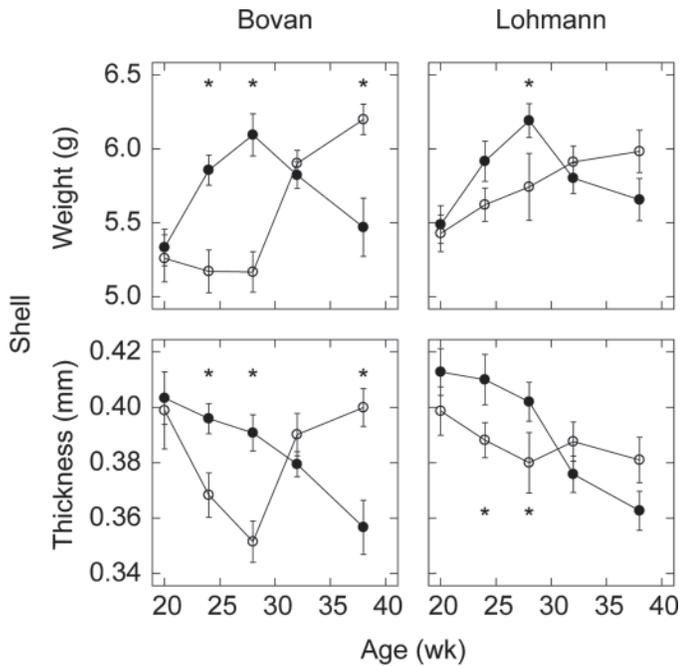
We also assessed the immunocompetence of the free-range hens by examining PHA response (Cheng and Lamont, 1988; Kean and Lamont, 1994; Smits et

al., 1999) and found that it was unrelated to parasite load. This result is perhaps not surprising given that the PHA assay assesses the cell-mediated immune response. Previous studies have tried to connect the PHA response to intestinal nematode loads, with contradictory results. The PHA response of Red Jungle Fowl (*Gallus gallus*) parasitized by *Ascaridia galli* was lower than that of nonparasitized birds (Johnsen and Zuk, 1999). In contrast, the immune responsiveness of red grouse (*Lagopus lagopus*) was not correlated with *Trichostrongylus tenuis* load, although the response was increased when the parasitic infection was experimentally reduced (Mougeot and Redpath, 2004).

No difference in BW was found between BB and LB hens. Interestingly, hens fed a DE-supplemented diet maintained their BW throughout the laying season whereas those on the control diet experienced a de-

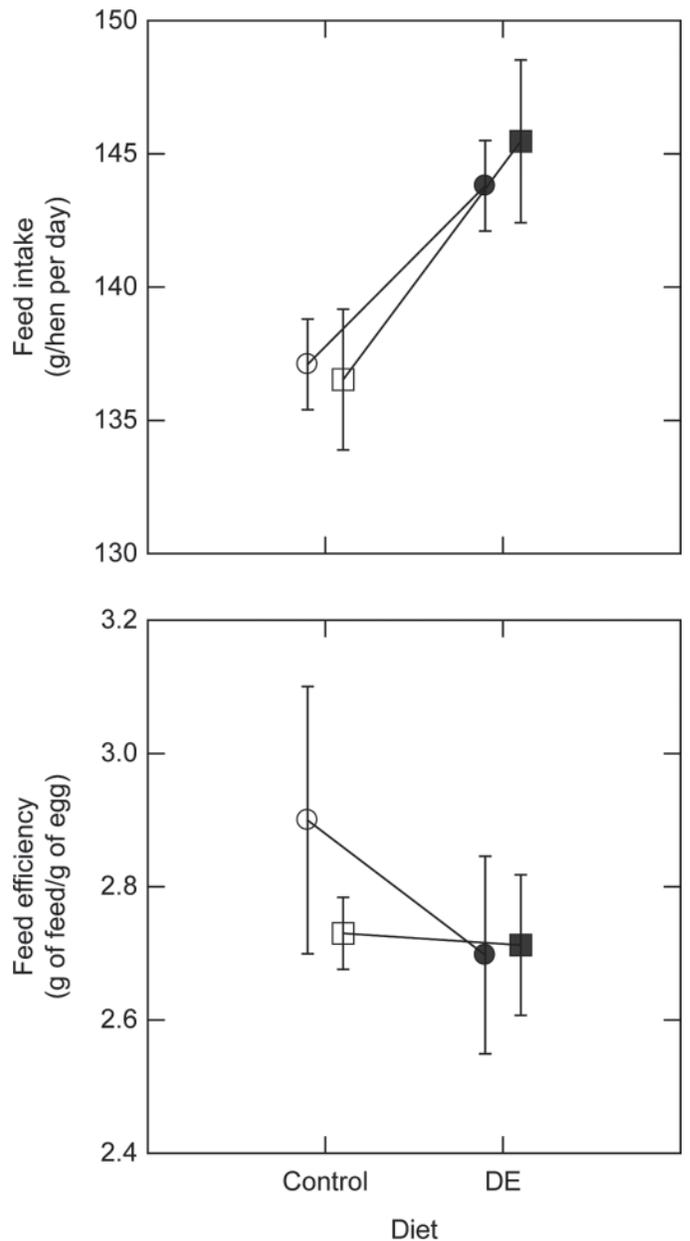


**Figure 8.** Effect of dietary diatomaceous earth on egg weight and weight of albumin and yolk of eggs produced by free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (left) and Lohmann Brown (right), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).



**Figure 9.** Effect of dietary diatomaceous earth on shell weight and thickness of eggs produced by free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (left) and Lohmann Brown (right), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth. Significant differences ( $P < 0.05$ ) between treatments are indicated by asterisks (\*).

crease in BW. This was found more in BB hens than in LB hens. In addition to having heavier BW, hens supplemented with DE consumed  $8 \pm 2$  g/hen per day more and laid more and bigger, better quality eggs, with a 92% hen day production, compared with 81% for the control hens during the laying season. It is not uncommon for free-range hens to lose weight when they start laying eggs (J. P. Jacob, University of Kentucky; personal communication), especially in situations in which they were moved to a new area for the laying season. Increased soil intake (estimated to be 14–32 g/d for free-range hens; van der Meulen et al., 2008) could be a major source of nutrient diluent. van der Meulen et al. (2008) reported that hens increased their feed intake in response to increasing amount of sand in their diet. This compensation allowed them to maintain their egg production and egg weight but BW gain was still compromised. It is not clear how DE could help hens to maintain their BW and better egg production. Hens fed the DE-supplemented diet consumed more and the increased feed intake could be a significant factor. It may be possible that DE offers essential trace elements or may improve absorption of nutrients. Diatomaceous earth consists of 86 to 94% silica, with the remainder containing alumina, calcium, phosphorus, sodium, potassium, magnesium, iron, sulfur, and other trace elements (Korunic, 1998; Mclean et al., 2005). The use of absorbent clay supplements in the form of phyllosilicates, such as bentonite and kaolinite, has been shown to have some direct benefits in poultry by improving



**Figure 10.** Effect of dietary diatomaceous earth on feed intake and feed efficiency of free-range organic laying hens. Two breeds of commercial brown-egg-laying hens, Bovan Brown (circles) and Lohmann Brown (squares), were fed a certified organic layer mash supplemented with (filled symbols) or without (open symbols) diatomaceous earth.

feed efficiency (Quisenberry, 1967). It has been suggested that these compounds increase the absorption of nutrients by slowing gastric passing (Quisenberry, 1967; Mallet et al., 2005). Therefore, perhaps DE also slows gastric passing and may increase absorption of nutrients that may prevent weight loss in hens during high egg production. One also must consider that, besides DE, the Red Lake Earth supplement contains 35% montmorillonite. Chemically, montmorillonite is hydrated sodium calcium aluminum magnesium silicate. For internal use, montmorillonite is effective in the treatment of irritable bowel syndrome (Ducrotte et al., 2005) and for the prevention of aflatoxicosis (Phil-

lips et al., 2002). Its copper bearing form has also been shown to improve growth performance in piglets and farm tilapia (Hu et al., 2005; Xia et al., 2005) and effectively remove lead from ingested contaminated feed in pigs (Yu et al., 2006). The combination of DE and montmorillonite may therefore improve the general health of the free-range hens and indirectly boost their resistance to internal parasites.

Based on the current price of organic table eggs, the cost of certified organic poultry feed, and the cost of the Red Lake Earth supplement, balancing the increased feed cost with the increase in egg sales, we estimated that supplementing DE in the diet increased the profitability of BB hens by \$0.06/hen per day and LB hens by \$0.03/hen per day.

Future studies may want to consider experimentally induced infections with consistent dosages of parasite eggs to analyze the effects of DE on *Eimeria*, *Ascaridia*, *Heterakis*, and *Capillaria* species in a laboratory setting. It would also be worthwhile to separate the effects of DE and montmorillonite in the diet.

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