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ORIGINAL ARTICLE

The influence of *Dermanyssus gallinae* and different lighting regimens on selected blood proteins, corticosterone levels and egg production in layer hens

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Abstract

Egg production in battery cage systems in commercial poultry farms promotes uncontrolled growth of poultry ectoparasite *Dermanyssus gallinae*. Intermittent lighting regimens provided a promising alternative for controlling *D. gallinae* invasions. The study analysed the influence of *D. gallinae* invasions on selected blood protein fractions (albumin, α-, β-, γ-globulin), corticosterone levels and egg production in Hy-Line Brown layer hens exposed to two lighting regimens: A (16 L:8D) and B (intermittent (4 L:2D); L-light, D-dark). Blood samples were collected from a total of 48 hens (divided into uninfested - UF, and infested - IF groups for each lighting regimen). The concentrations of protein fractions were analysed by electrophoresis on Cormay Gel Protein 100, and corticosterone levels were determined in a radioimmunoassay. The results of the study revealed concentrations of β-globulin and corticosterone levels were significantly higher in IF than UF groups in both lighting regimens. However, both parameters were higher in hens exposed to lighting regimen B than lightening regimen A. Gamma-globulin concentrations were significantly lower in IF than UF groups in both lighting regimens. Egg production was significantly lower in all groups than commercial standard. *D. gallinae* and intermittent lightening regimen had interaction effect on the corticosterone level in hens. Strong decreasing (negative) linear relationship between corticosterone levels and egg production ($r = -0.911$) was reported.

Keywords Haematophagous parasites · Blood protein · *D. gallinae* · Layer hens · Lighting regimen · Stress

Introduction

Dermanyssus gallinae (De Geer, 1778) (*D. gallinae*) commonly known as poultry red mite (PRM) is a temporary, haematophagous parasite of birds (Chmielewski 1982; Roy and Chauve 2007). The mite is widely dispersed throughout the world and is a major problem in commercial poultry production, mainly in laying hens flocks. *D. gallinae* invasions have been reported in different systems of rearing: battery cage, barn, free-range, backyard and organic poultry breeding systems (Sparagano et al. 2009). It is estimated that losses caused by the invasion of *D. gallinae* and eradication costs in the EU amount to about EUR 130 million annually (Van Emous 2005). *Dermanyssus gallinae* acts as a micropredator and feeds on the blood mainly at night. During the daytime, the parasite leaves the host and reproduces in cracks and crevices of the poultry house (Wood 1917; Nakamae et al. 1997; Chauve 1998; Sokół and Romaniuk 2007). Battery cage system promotes uncontrolled proliferation of *D. gallinae* due to high constant temperature and relative humidity, a large number of hosts in limited space, the presence of numerous cracks and gaps in cage structure and a long production cycle that lasts 80–90 weeks (Roy et al. 2010). In hideouts, *D. gallinae* is protected from chemical control agents and is difficult to combat (Chauve 1998; Sokół and Romaniuk 2007). The introduction of enriched cages (EU Directive 1999.74/EC) has contributed to significant and uncontrolled proliferation of *D. gallinae* (Chirico and Tauson 2002; Sparagano et al. 2009). Battery cage systems are the predominant form of housing for laying hens on most commercial poultry farms: Denmark −56%; France - 76.5%; and Italy - 96.4% (Sparagano et al. 2009; Harrington et al. 2011). Hens infested

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by the poultry red mite show distress, poor feed conversion and decreased egg production and inferior shell quality (Chauve 1998; Pilarczyk et al. 2004; Mul et al. 2009). Pale mucous membranes, anaemia resulting from significant blood loss, and increased mortality were observed in infested flocks (Kirkwood 1967; Wójcik et al. 2000; Cosoroaba 2001; Cencek 2003; Van Emous et al. 2005). The PRM can also infect humans and mammals and causes pruritus and skin lesions (Auger et al. 1979; Rosen et al. 2002; Mignon and Losson 2008; Akdemir et al. 2009; Haag-Wackernagel and Bircher 2010).

Chemical synthetic acaricides commonly applied to control *D. gallinae* invasions have certain limitation. Failures (Zeman and Zelezny 1985; Beugnet et al. 1997; Chauve 1998) and resistance in mites has been reported (Fiddes et al. 2005). Many alternative solutions including: natural substances (toxin from *Bacillus thuringiensis*, spinosad, garlic extract, neem tree extract, geraniol, eugenol and citral), vaccinations and biological control of natural enemies (Bartley et al. 2017; Beugnet et al. 1997; Chauve 1998; Maurer and Perler 2006; Stafford et al. 2006; Sokół and Romaniuk 2007; Kilpinen and Steenberg 2009; Mul et al. 2009; Sparagano et al. 2009) has been developed however new effective controlling method are still being sought. Intermittent lighting regimens widely used in commercial poultry farms to increase productivity, improve egg shell quality, and reduce mortality and cannibalism (Lewis et al. 1992) has been reported to be a promising alternative method for controlling *D. gallinae* (Zoons 2004; Stafford et al. 2006). The mode of action of the shorten dark phase is to disturb the feeding patterns of *D. gallinae,* confine parasites to hideouts, limit reproductive potential and prevent from infecting hosts. Our recent study revealed different conclusions that intermittent lighting regimens could encourage *D. gallinae* to move constantly and that the stress caused by frequent light change supports parasitic reproduction (Sokół et al. 2008). What is more *D. gallinae* invasions can cause somatic and psychogenic stress in layer hens, which compromises humoral immunity and egg performance (decrease in the blood levels of γ-globulin and decreased laying performance) (Kowalski and Sokół 2009). Thus, it can be hypothesized that *Dermanyssus gallinae* infestation and lighting regimen may affect hen stress status alone and in combination.

The aim of this study was to analyse the influence of a natural *D. gallinae* invasion on the blood levels of albumin, α-, β-, γ-globulins (health and immunity status parameters), corticosterone (stress hormone) and egg production in Hy-Line Brown layer hens exposed to two lighting regimens: A – continous, consisted of 16 h of light (L) followed by 8 h of darkness (D) per day (16 L:8D), and B- intermittent, consisted of periods of 4 h of light and 2 h of darkness periods following alternately during 24 h (4 L:2D - intermittent lighting regimen).

Materials and methods

Animals

The study was performed on a total of 48 Hy-Line Brown layer hens randomly sampled from 2 poultry houses of a one commercial poultry farm implemented two different lighting regimens. Twenty-four hens were sampled from poultry house No. 1, and 24 hens – from poultry house No. 2 (see Pre-experimental conditions). The hens were transported to the animal house of the Faculty of Veterinary Medicine at the University of Warmia and Mazury in Olsztyn, and divided into experimental groups (see Experimental conditions).

The experiment was carried out in compliance with the recommendations of the Local Ethics Committee for Animal Experimentation in Olsztyn (no. 59/2006).

Pre-experimental conditions

In poultry house No. 1, hens were exposed to continuous lighting regimen consisting of 16 h of light followed by 8 h of darkness per day (16 L:8D). In poultry house No. 2, hens were exposed to intermittent lighting regimen consisting of alternating periods of 4 h of light and 2 h of darkness per day (4 L:2D). Light intensity during the light phase (10 lx according to the recommendations of the Illuminating Engineering Society of North America, 2001), environmental conditions, feed and water access were identical in both poultry houses. Hens were naturally infested with *D. gallinae*. The level of infestation was monitored using the method previously described by Sokół and Romaniuk (2007). On the sampling day, 1 g of debris obtained from each poultry house contained around 2000 developmental forms of the parasite (30% adult females, 45% larvae and nymphs, and 25% eggs). The collected material was indicative of severe red mite infestation (Sokół et al. 2008).

Experimental conditions

In the animal house, each group of 24 hens was divided into two subgroups of 12 birds: infested (IF, $n = 12$) uninfested (UF, n = 12) by *D. gallinae*. The UF group was obtained by eliminating *D. gallinae* by spraying hens, cages and rooms with Butox 50 (active ingredient deltamethrin) at a dose of 1 mL/1 L H_2O. The treatment was repeated after 4 days. Before the experiment, the presence of *D. gallinae* was inspected visually. During the experiment, each group of hens was kept in cages in separate rooms to avoid contamination of *D. gallinae.* The conditions and lighting regimens were identical to those applied in the poultry farm. Hens received feed from the poultry farm and water ad libitum. The adaptation period lasted 2 weeks, and the experiment lasted 4 weeks. On the last day of the experiment, blood samples were collected

for analyses. Light intensity during the light phase was 10 lx according to Council Directive 1999/74/EC (maximum of 15–18 h continuous light (10–25 lx) per day and a dark phase of minimum 8 h per 24 h).

Collection of blood samples

Hens were humanely euthanized by rapid decapitation at 42 weeks of age. Before decapitation, each bird was kept for 5 min in a dark box to ensure that the levels of the monitored parameters were not affected by stress induced by sample handling. Blood samples were collected within seconds into heparinized tubes (0.2 ml of heparin/10 ml of whole blood), centrifuged for 10 min at 4 °C and 1200 g. The obtained serum samples were stored at −70 °C until laboratory analysis. The following parameters were analysed 1.) immunological parameters: concentrations of albumins, α-, β- and γ-globulins, by agarose gel electrophoresis with Cormay Gel Protein 100 in the Cormay S-20 chamber according to the manufacturer's protocol (Cormay, Lublin, Poland), and the DS-2 densitometer; 2.) Corticosterone levels by the radioimmunoassay method developed by Kokot and Stupnicki (1985) using titrated corticosterone (Amersham) and antibodies developed by Szafrańska et al. (2002) at the Department of Animal Physiology, University of Warmia and Mazury in Olsztyn.

Monitoring egg production

Egg production was monitored for 4 weeks (in hens aged 39, 40, 41 and 42 weeks). The results were expressed as total weekly production and as a percentage of the commercial egg production standard for Hy-Line Brown hens on a weekly basis. Mean production during the study was also expressed as a percentage of the commercial standard for weeks 39–42 (92%). According to the information provided by the producer of Hy-Line Brown hens, egg production in weeks 39, 40, 41 and 42 should reach 93%, 92%, 92% and 91%, respectively, where 1 egg/23 h/hen represents 100% production capacity.

Statistical analyses

Results were statistically analysed by calculating mean value, standard deviation, and by using two-factorial analysis of variance (ANOVA); Tukey post-hoc test; and Pearson correlation coefficient between analyzed parameters. All effects were statistically significant at the $p < 0.05$. Statistical analyses were performed using STATISTICA Software.

Results

Concentrations of albumins, α-, β-, γ-globulins and corticosterone levels in the blood of hens uninfested (UF) and infested (IF) with *D. gallinae,* exposed to lighting regimens: A and B are presented in Table 1. Beta-globulin and corticosterone levels were significantly higher in IF groups than in UF groups regardless of the lighting regimen. Corticosterone levels were approximately 1.5-fold higher in the IF group than in the UF group exposed to lighting regimen A, and 2.5-fold higher in the IF group than in the UF group exposed to lighting regimen B and 4-fold higher in IF B than in the IF A group. The concentration of γ-globulin was significantly lower in IF groups than in UF groups regardless of the lighting regimen. Results show interaction effect of *Dermanyssuss galliane* and intermittent lighting regimen B on corticoserone level in IF B group.

Egg production in hens uninfested (UF) and infested (IF) with *D. gallinae* and exposed to lighting regimens A and B is presented in Table 2. Table shows weekly egg production in hens in the 4-week study and the percentage of egg production compared with standard egg production for hens recommend by the producer of Hy-Line Brown hens (egg production in weeks 39, 40, 41 and 42 should reach 93%, 92%, 92% and 91%, respectively, where 1 egg/23 h/hen represents 100% production capacity). In all investigated groups, egg production was significantly lower than the commercial standard. Uninfested hens exposed to lighting regimen A produced 0.67 egg/ day (27.6% less than the standard), and IF hens produced 0.60 egg/ day (34.3% less than the standard). Uninfested hens exposed to lighting regimen B produced 0.62 egg/ day (32.4% less than the commercial standard), IF group produced 0.53 egg/ day (41% less than the commercial standard).

The results of the study revealed a strong decreasing (negative) linear relationship between corticosterone levels and egg production ($r = -0.911$), a strong increasing (positive) linear relationship between corticosterone and globulin β levels ($r = 0.865$), a moderate decreasing (negative) relationship between corticosterone and globulin γ levels ($r = -0.423$), a strong decreasing (negative) linear relationship between egg production and globulin β levels ($r = -0.954$), a strong increasing (positive) linear relationship between egg production and globulin γ levels ($r = 0.705$), and a moderate increasing (positive) relationship between globulin γ and globulin β levels ($r = 0.539$).

Discussion

The study showed an increase in levels of the corticosterone and β-globulin; and decrease in levels of γ- globulin and egg production in hens infested with *Dermanyssus gallinae* comparing with hens non infested. However, corticosterone and β-globulin levels were higher in hens exposed to intermittent lightening regimen B (4 L:2D) than lightening regimen A. What is more the influence of *D. gallinae* in the combination

Table 1 Concentration of albumins, α-, β-, γ-globulin, and corticosterone level in blood of hens uninfested (UF) and infested (IF) by *D. gallinae,* exposed to two different lighting regimens (A 16 L:8D and B 4 L:2D)

Lighting regimen/Group		Albumins (%)	Globulins (%)			Corticosterone (μg/ml)
			α	β	γ	
A	UF	44.27 ± 5.80	5.25 ± 1.25	10.19 ± 1.51^{a}	40.29 ± 5.07^{b}	1.88 ± 0.45^{a}
	IF	44.29 ± 3.11	5.49 ± 1.18	15.14 ± 3.40^{b}	34.98 ± 3.32^{a}	2.54 ± 0.42^{b}
B	UF	37.87 ± 1.8	5.46 ± 0.63	15.94 ± 0.81^{b}	40.73 ± 1.6^{b}	3.47 ± 0.15^{c}
	IF	39.34 ± 1.8	5.24 ± 1.85	19.53 ± 2.41^{c}	35.89 ± 2.4^{a}	8.13 ± 0.52^{d}

Differences significant at $p \leq 0.05$

L - light phase, D dark phase (hours)

with intermittent lighting regimen B (4 L:2D) had interaction effect on the corticosterone level in the IF B group. Strong decreasing (negative) linear relationship between corticosterone levels and egg production (r = −0.911) was reported.

Corticosterone is a stress hormone produced by the adrenal cortex in response to stimulation of the hypothalamic-pituitary-adrenal axis. The level of corticosterone in the blood indicates the degree of somatic stress in birds (Campo et al. 2008). The study revealed approx. 1.5-fold significant increase in the level of corticosterone in the IF group than in the UF group exposed to lighting regimen A; and 2.5-fold increase in the IF group than UF group exposed to lighting regimen B; and 4-fold increase in IF B than in the IF A group. All results were significant at $p < 0,05$. These results indicates that *Dermanyssus gallinae* and intermittent lighting regimen B consisted of 4 L:2D had positive influence on the corticosterone levels alone and interaction effect in the combination (Table 1). The impact of *Demanyssus gallinae* infestation on hens stress status was described previously (Kowalski et al. 2006; Kowalski and Sokół 2009). The negative impact of the intermittent lighting regimen B may be caused by disruption of the natural circadian rhythm of hens. The use of light cycles with a dark phase shorter than 8 h per 24 h is banned currently by the EU directive on animal welfare. The interaction effect of *Dermanyssuss galliane* and intermittent lighting regimen B on the increased corticoserone level can be explained by the hypothesis that *D. gallinae* feeding behaviour on hens is stimulated and exacerbated by intermittent lighting regimen. This hypothesis is supported by the results of our previous experiment (Sokół et al. 2008) investigated the influence of lighting regimens (16 L:8D and 4 L:2D) on the behaviour of *D. gallinae*. In the cited experiment, all female parasites deposited a total of around 12 eggs/h during the light phase of the 16 L:8D cycle, and around 70 eggs/h during the light phase of the 4 L:2D intermittent lighting regimen. In the dark phase, all females deposited of a total of around 14 eggs/h and 132 eggs/h, respectively (Sokół et al. 2008).

Blood serum proteins play numerous roles in animal physiology and are important indicators of the animals' health status (Azim et al. 2004; Tothova et al. 2016). Albumin is the largest blood protein fraction and major reservoir of blood proteins. Regulates osmotic pressure and the acid-base balance, and it is responsible for the transport of vitamins, minerals, hormones and fatty acids (Mackiewicz 1997; Hankins

Table 2 Egg production in hens uninfested (UF) and infested (IF) by *D. gallinae,* exposed to two different lighting regimens (A 16 L:8D and B 4 L:2D)

Lighting regimen/Group		Age of hens (weeks)				Mean number of eggs/day/hen (mean % of egg production)
		39	40	41	42	
		% of egg production acc. to producer				
		93%	92%	92%	91%	
		Number of laid eggs (% of egg production)				
A	UF	57	56	56	57	0.67^{c}
		(65.3%)	(64.1%)	(64.1%)	(65.3%)	(64.4%)
	IF	52	52	52	53	0.60^{b}
		(59.5%)	(59.5%)	(59.5%)	(60.7%)	(57.7%)
B	UF	51	52	52	52	0.62^{b}
		(58.4%)	(59.5%)	(59.5%)	(59.5%)	(59.6%)
	IF	42	47	47	48	0.53^{a}
		(48.1%)	(53.8%)	(53.8%)	(54.9%)	(51.0%)

Differences significant at $p \leq 0.05$

L - light phase, D - dark phase (hours)

2006). Alpha-globulin are fraction produced almost exclusively in the liver, indicates infectious diseases and chronic inflammations. Alpha-globulin levels increase in acute nephritis, severe active hepatitis, active systemic inflammation, malnutrition and nephrotic syndromes. Their levels decrease in hepatic insufficiency, severe inanition, blood loss and protein-losing gastrointestinal disorders (Gabay and Kushner 1999). The γ- globulin fraction includes immunoglobulins (antibodies) responsible for immunity against disease (Jackson and Elsawa 2015). Investigated variables had significant influence on the beta-globulin level alone and in the combination had additive effect (Table 1.) The gamma-globulin level was the same in both IF groups. These results indicate that *D. gallinae* infestation had influence to impair humoral immunity in investigated hens and there was no interaction effect with lighting regimen. In this study, the increase in corticosterone levels in investigated groups was correlated with a significant increase in the concentrations of β-globulin and a significant decrease in the concentrations of γ-globulin. Our previous study revealed a similar increase in corticosterone levels and a decrease in γ-globulin concentration in hens infested with *D. gallinae* (Kowalski et al. 2006; Kowalski and Sokół 2009).

Corticosterone secreted during the stimulation of the HPA axis may also suppress the hypothalamic-pituitary-gonadal axis which regulates egg production (Kowalski et al. 2006; Kowalski and Sokół 2009). According to the information provided by the producer of Hy-Line Brown hens, egg production in weeks 39, 40, 41 and 42 should reach 93%, 92%, 92% and 91%, respectively, where 1 egg/23 h/hen represents 100% production capacity. In our study, egg production in all groups was below the commercial standard and was lower in groups exposed to regimen B than in groups exposed to lighting regimen A (Tale 2.) The study demonstrated decreasing (negative) linear relationship between corticosterone levels and egg production ($r = -0.911$). The literature data shows a red mite invasion decrease egg production by 10% (Wójcik et al. 2000), and by 15–20% (Pilarczyk et al. 2004).

Different types of the intermittent lighting regimen have been reported to significantly limit the number of *D. gallinae* without inducing a significant drop in egg production. Zoons (2004), revealed that *D. gallinae* were more effectively controlled under an intermittent lighting regimen (15 min of light and 45 min of darkness followed alternatively (15'L:45'D) than under a regimen consisting of 16 intermittent 15'L:45'D cycles, followed by 8 h of darkness. Similar results were reported by Stafford et al. (2006) who explained the reduction in the number of *D. gallinae* by the fact that poultry red mite leaving the host and visible during the light phase may be eaten by hens. The stress status of investigated hens in our experiment may suggest that hens may be attacked by mites more frequent. In our experiment, we used 10 lx light intensity during the light phase (standard lighting for poultry farms) while Stafford used 86 lx of light intensity. We concluded that light intensity of 10 lx during the light phase is probably insufficient to control the invasion by disturbing feeding behaviour of *D. gallinae* and confine red mites to their hideouts.

Blood serum proteins play numerous roles in animal physiology and are important indicators of the animals' health status. Corticosterone level is robust markers of somatic stress in layer hens. Our findings revealed that *D. gallinae* infestations increase the concentration of the stress hormone corticosterone, compromise immunity and decrease egg production in layer hens. What is more *D. gallinae* infestation in the combination with intermittent lightening regimen can lead to exacerbate the level of stress in hens. An intermittent lighting regimen consisting of alternating periods of 4 h of light and 2 h of darkness per day (4 L:2D) with light intensity of 10 lx during the light phase is not effective in controlling *D. gallinae* invasions. Intermittent lightening regimen in heavily infested hen houses may exacerbate feeding behaviour of *D. gallinae* which lead to increase the level of corticosterone and affects egg production in hens.

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Compliance with ethical standards

Statement on the welfare of animals All procedures performed in studies involving animals were in accordance with the ethical standards of the Local Ethics Committee for Animal Experimentation in Olsztyn (no. 59/2006).

Conflict of interest statement The authors declare that they have no conflict of interest.

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