Polish Journal of Veterinary Sciences Vol. 12, No. 2 (2009), 231-235

Original article

Influence of *Dermanyssus gallinae* (poultry red mite) invasion on the plasma levels of corticosterone, catecholamines and proteins in layer hens

A. Kowalski, R. Sokół[1]

Division of Pathophysiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland
[1] Division of Parasitology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland

Abstract

The results of studies conducted in 2006 revealed that mass red mite (*Dermanyssus gallinae)* invasions cause somatic stress which may be responsible for the pathophysiological mechanism of decreased egg production, lower humoral immunity and higher mortality in layer hens. The aim of this study was to validate the above research results, to investigate whether in addition to somatic stress, red mite invasions cause psychogenic stress due to the activation of the sympatho-adrenomedullar system, and to determine the level of stress resulting from red mite infestations in comparison with a short, 1.5 h period of acute immobilisation stress.

The study investigated 36 HY-Line Brown layer hens divided into three groups: a non-infested control group, an experimental group infested with red mites and a non-infested experimental group subjected to acute immobilisation stress for 1.5 h. Blood samples were taken from all hens for the determination of the levels of corticosterone, adrenaline, noradrenaline, albumin, and α-, β- and γ-globulins.

The results validated the previous reports on the occurrence of somatic stress and on a significant decrease in γ-globulin levels ($p \leq 0.01$) in the group of birds infested with red mites, in comparison with the control group. Adrenaline levels in infested hens were indicative of psychogenic stress. Based on a comparison of hormonal indicators in all hen groups, the level of somatic stress resulting from red mite infestation can be classified as moderate, while the level of psychogenic stress can be interpreted as high. A significant drop in γ-globulin levels in the blood of birds infested with red mites also shows that the invasion induces chronic stress which lowers the humoral immunity of hens.

Key words: *Dermanyssus gallinae*, poultry red mite, laying hens, stress

Correspondence to: A. Kowalski, tel: +48 089 523 32 96, fax: 48 089 523 34 40, e-mail: ankow@uwm.edu.pl

Introduction

Mass red mite *(Dermanyssus gallinae)* infestations pose a serious economic, sanitary and animal welfare problem in many poultry farms across Europe. The above applies mainly to layer hens in commercial poultry farms with a high bird density within a relatively restricted space. In poultry farms infested with red mites, layer hens show weight loss, increased feed intake, behavioural disorders, a higher disease incidence, decreased egg production and an increase in mortality rates to 6-8% (Pilarczyk et al. 2004). To date, research studies have not provided a satisfactory explanation as regards the pathophysiological mechanism which is responsible for the above disorders, in particular a decrease in egg laying, and which contributes significantly to hen mortality at various development stages of *Dermanyssus gallinae* infestation. The relevant publications and research results address more detailed aspects of the problem, such as the biology of the red mite life cycle and development (Nordenfors et al. 1999, Nisbet and Billingsley 2000, Bruneau et al. 2001, Kilpinen 2001, Cruz et al. 2005), pathogenic microflora carrier states (Reshetnikov 1967, Petrov 1975, Chirico et al. 2003) red mites as a source of vector infections in animals and humans (Skierska 1968, Daniel and Cerný 1971, Ramsay et al. 1975, Beck 1999, Rosen et al. 2002, Haag-Wackemagel 2005), and effective methods of preventing red mite infestations (Nordenfors and Höglund 2000, Soon-Il et al. 2004). According to scant studies on the subject, blood-feeding red mites could also be responsible for moderate anaemia in hens, but the authors of such papers do not support the above argument as the root cause of high mortality rates in layer farms (Kirkwood 1967, Jungmann et al. 1970, Pilarczyk et al. 2004, Kilpinen et al. 2005).

Behavioural observations conducted during massive red mite invasions have shown that layer hens were in a state of heightened irritation and anxiety which was manifested by increased behavioural responses, such as excessive sensitivity to environmental stimuli, enhanced motor activity, vocalisation and self-grooming (Turner et al. 2003, Pilarczyk et al. 2004, Kilpinen et al. 2005). The stress-inducing influence of *Dermanyssus gallinae* on hens results from the feeding mechanism of the parasites which dwell massively on the birds and suck their blood. Changes in the behaviour of infested hens could be indicative of moderate chronic stress which activates the hypothalamic-pituitary-adrenocortex axis (Ladewig 1987). Its hormones have a suppressive effect on the hypothalamic-pituitary-gonadal axis which conditions egg laying (Oliverio 1987, Rivier and Rivest 1991). A heightened hormonal stress response may be limited by increased behavioural activity (Dantzer and Mormede 1983, Dantzer 1986). Nevertheless, stress may result in physiological and pathomorphological changes which are difficult to detect, leading to a deterioration of the general health of the birds, systemic resistance, and egg laying capacity, and increased mortality rates (Armario et al. 1986). This "stress" hypothesis was validated by a study (Kowalski and Sokół 2006) which showed a significant increase in corticosterone levels and a decrease in gamma globulin levels in the blood of hens massively infested with red mites. The authors of this study did not, however, report anaemia or skin inflammations in infested birds.

Since the above experiment was the only one to account for the somatic stress hypothesis, the objectives of the present study were to: 1. validate the previous results with respect to layer hens from a different farm with a similar infestation level, 2. investigate whether, in addition to somatic stress, red mite infestation causes psychogenic stress due to heightened stimulation of the sympatho-adrenomedullar system, 3. determine the level of stress induced by red mite invasion in comparison with a short period of acute immobilisation stress.

Materials and Methods

The study investigated 36 HY-Line Brown layer hens weighing 1.8-1.9 kg, at 51 weeks of age and 35 of the laying season, divided into three groups (of 12 birds each): 1 – non-infested control group, 2 – experimental group infested with *Dermanyssus gallinae*, 3 – non-infested experimental group subjected to acute immobilisation stress for 1.5 h.

Prior to the experiment, the birds were kept in litterless battery cages, measuring 0.5 x 0.61 x 0.41 m, 6 hens per cage, in a house populated by approximately 85000 layer hens. They were automatically fed farm-made feed and had free access to water. Feed supply, egg collection and waste removal were mechanised. In the hen house where the experimental birds were kept, red mite infestation was monitored on a weekly basis with the use of paper trap tubes positioned on the floor and under the cages. Although the hen house had been subjected to a red mite protection program prior to stocking, the level of infestation with the investigated parasite increased gradually in the farm and was described as high at the beginning of the experiment (around 300 red mite females were determined in 100 g of the sediment sampled from the floor and from under the cages). The red mite concentration was determined by counting females in the collected and cooled sediment, emptied on a Petri dish with marked fields of 2 x 2 cm, under an Olympus SZX12 magnifying glass.

Experimental birds were randomly sampled from different cages and transported to the animal house of

the Parasitology Unit, Faculty of Veterinary Medicine in Olsztyn, where local infectious and stress factors (conveyor belt noise, ventilation hum, high bird density) other than red mite infestation were eliminated. Each bird group was placed in three separate compartments. Prior to transport to the animal house, the control group and the immobilised group hens were dusted with Butox (deltamethrine) in a 1 ml/1l H_2O solution to eliminate parasites. These hens were repeatedly dusted with the same solution 3 days after placement in the animal house. After 3 days these double dusted hens were examined to ensure that they were free of parasites.

Separate transportation was provided for birds infested and non-infested with the poultry red mite. During the experiment, the hens were kept in cages identical to those on the poultry farm, 6 birds per cage, for 10 days. They were fed farm-made feed and had free access to water. The hens were subjected to the same light program (light phase from 7 a.m. to 11 p.m.) and their behaviour was monitored.

After 10 days, one group of 12 hens was killed daily by decapitation at the same time of day (9.00-10.30 a.m.) to avoid daily fluctuations in the level of adrenocortical hormones (Davis and Siopes 1989). Prior to slaughter, each bird was kept in a dark box measuring 0.6 x 0.6 x 0.6 m to reduce manipulative stress which might have distorted the physiological levels of the investigated stress hormones. Hens were bled within several seconds and blood samples were collected. Blood was sampled and collected in heparinised test tubes cooled in ice water (0.2 ml of heparin per 10 ml of whole blood). The collected and rapidly cooled blood samples were centrifuged for 10 min. at 4°C and 1200 g, and the blood plasma obtained was freeze-stored at -74°C until laboratory analysis. The group 3 birds were subjected to acute immobilisation stress by fixing the legs for 1.5 h between 7.30-9.00 a.m. They were then kept in a dark box for 5 minutes and were then decapitated and blood samples collected.

The plasma levels of the following hormones were determined: corticosterone – by the radioimmunological method (Kokot and Stupnicki 1985) with the use of tritiated corticosterone supplied by Amersham and antibodies produced by the Division of Animal Physiology of the University of Warmia and Mazury in Olsztyn by the method proposed by Szafrańska et al. 2002, adrenaline and noradrenaline – by the HPLC standard method with the use of an HP 1049A electrochemical detector, albumins, α, β and γ globulins – by agarose gel electrophoresis with a standard Cormay Gel Protein 100 set (produced by P.Z. Cormay, Lublin, Poland) in a Cormay S-20 electrophoretic chamber and DS-2 densitometer.

Statistical analysis

The results are presented as arithmetic mean values and standard deviations ($x \pm SD$). The differences were verified with Student's t-test by comparing the values obtained in the experimental groups against the control group and between the two experimental groups, at a significance level of $P \leq 0.05$ and $P \leq 0.01$ (Statgraphics Statistical Graphics System, v. 2.6).

Results

A state of heightened behavioural reactivity to external stimuli, caused by polyetiologic stress which is typical of hens raised in farms with a high bird density, was observed in all birds until the third day following transfer to the animal house. On the following days, increased self-grooming activity, a characteristic symptom of anxiety, was reported only in the group of birds infested with red mites (group 2).

The results of laboratory analyses are presented in Table 1. The reported blood serum protein fractions showed a significant decrease in γ-globulin levels and an increase in β-globulin levels in hens infested with red mites ($p \leq 0.01$), suggesting that the humoral immunity of the birds had been impaired. Significant differences in serum corticosterone levels were determined between all investigated bird groups. In comparison with the control group, the corticosterone levels were nearly 1.5 times higher ($p \leq 0.01$) in the infested group and nearly 3 times higher ($p \leq 0.01$) in the group of birds subjected to stress for 1.5 h. Psychogenic stress indicators (catecholamine) showed a significant increase ($p \leq 0.01$) in adrenaline levels in the experimental groups, while a growing trend (statistically non-significant) in noradrenaline levels was noted in the group of birds infested with red mites.

Discussion

Behavioural observations indicating anxiety in all hens (increased vocalisation and motor activity) until the third day of the experiment were indicative of a standard adaptive response caused by 1 hour of transport and stress induced by a new breeding environment. As regards group 2 birds (infested with red mites), a state of moderate stimulation reported until the end of the observation period (day 10), noted also by other authors (Kilpinen 2001, Pilarczyk et al. 2004, Kilpinen et al. 2005), was manifested by increased vocalisation and self-grooming due to the

Table 1. Results of analyses to determine blood serum protein fractions, catecholamine and corticosterone levels in the blood of layer hens (x ± SD, n = 12).

Indicator	Unit of measure	Group 1 (control) n = 12	Group 2 (experimental, infested with red mites) n = 12	Group 3 (experimental, subjected to 1.5 h immobilisation stress) n = 12
Albumins	g/dl	1.81 ± 0.24	1.82 ± 0.13	1.82 ± 0.31
α globulins	g/dl	0.22 ± 0.05	0.23 ± 0.05	0.23 ± 0.06
β globulins	g/dl	0.42 ± 0.06	0.82 ± 0.14**	0.43 ± 0.12*
γ globulins	g/dl	1.65 ± 0.10	1.23 ± 0.11**	1.62 ± 0.16
Adrenaline	ng/ml	9.73 ± 3.94	22.53 ± 9.65**	17.26 ± 9.20*[11]
Noradrenaline	ng/ml	3.72 ± 2.21	8.19 ± 7.70	4.23 ± 1.32
Corticosterone	ng/ml	1.88 ± 0.45	2.54 ± 0.42**	5.13 ± 1.19**[11]

*, ** – Key: statistically significant differences at $p \leq 0.05$ and $p \leq 0.01$ between the experimental groups and the control group (1 – 2, 1 – 3).
[1], [11] – Key: statistically significant differences at $p \leq 0.05$ and $p \leq 0.01$ between the experimental groups (2 – 3).

stress induced by the parasites feeding on the hens and causing skin irritation. The above is supported by the results of hormone level analyses. In comparison with the control group, a statistically significant increase in the levels of circulating plasma corticosterone, adrenaline and a growing trend in noradrenaline levels in the infested hens clearly indicates a stress reaction in those birds. The hormonal indicators applied, which represent the end products of activation of the hypothalamic-pituitary-adrenocortex axis (corticosterone) and the sympatho-adrenomedullar system (adrenaline and noradrenaline), indicate the development of somatic and psychogenic stress reactions caused by the studied ectoparasites, following the elimination of other environmental stress factors which are characteristic of farm breeding. The observed 1.5-fold increase in corticosterone levels in HY-Line Brown hens was lower than in Lowman Brown birds, where it rose by nearly 2.5 times (Kowalski and Sokół 2006). The above could suggest that HY-Line Brown hens are less sensitive to stress or that their susceptibility to stress decreases with age at a similar intensity of red mite infestation since the Lowman Brown hens were younger (27 weeks of age, week 11 of the laying season, compared to the HY-Line Brown hens -51 weeks of age, week 35 of the laying season). A significant drop in γ-globulin levels in hens infested with red mites supports a well-known mechanism of immunity suppression under stress, in particular chronic somatic stress, in consequence of which the adrenocorticotropin hormone and adrenal steroids have a destructive impact on neutrophil and lymphocyte counts (B limphocytes are the source of γ-globulins), leading to changes in their mutual quantitative proportions in the blood (Quan et al. 1995, Prabhakaran et al. 1997).

A comparison of hormone levels in the group of hens infested with red mites and birds subjected to acute immobilisation stress for 1.5 h supports the conclusion that parasite-induced stress moderately stimulates the hypothalamic-pituitary-adrenocortex axis and strongly activates the sympatho-adrenomedullar system. The above could offer an explanation for the pathophysiological mechanism responsible for a significant drop in γ-globulin levels which makes infested hens more susceptible to infectious factors, as well as changes in behaviour, lower egg production and increased mortality in hen flocks during a mass red mite (*Dermanyssus gallinae*) infestation.

Conclusions

The results of the study confirmed the increased stimulation of the hypothalamic-pituitary-adrenocortex axis (somatic stress) and the high activity of the sympatho-adrenomedullar system (psychogenic stress) in layer hens infested with red mites.

Chronic stress may require follow-up research to investigate the pathological effect of mass red mite (*Dermanyssus gallinae*) infestation on suppressed immunity, lower egg production, a higher disease incidence, higher mortality and behavioural changes in layer hens raised in commercial poultry farms.

This study was financed by the Polish Committee for Scientific Research, research project No. 0531.0802, with the permission of the Committee of Ethics at the Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland: permit No. 59/2006.

References

Armario A, Lopez-Calderon A, Jolin T, Balasch J (**1986**) Response of anterior pituitary hormones to chronic stress. The specifity of adaptation. Neurosci Biobehav Rev 3: 245-250.

Beck W (**1999**) Landwirtschaftliche Nutztiere als Vektoren von parasitaren Epizoonoseerregern und zoophilen Dermatophyten. Hautarzt 50: 621-628.

Bruneau A, Dernburg A, Chauve C, Zenner L (**2001**) First in vitro cycle of the chicken mite, *Dermanyssus gallinae* (De Geer 1778), utilizing an artifical feeding device. Parasitology 123: 583-589.

Chirico J, Eriksson H, Fossum O, Jansson D (**2003**) The poultry red mite, *Dermanyssus gallinae*, a potential vector of Erysipelothrix rhusiopathiae causing erysipelas in hens. Med Vet Entomol 17: 232-234.

Cruz MD, Soler Vega Robles MC, Jespersen JB, Kilpinen O, Birkett M, Dewhirst S, Pickett J (**2005**) Scanning electron microscopy of foreleg tarsal sense organs of poultry red mite *Dermanyssus gallinae* (De Geer) (*Acari*: *Dermanyssidae*). Micron 36: 415-421.

Daniel M, Cerný V (**1971**) A case of a mass occurrence of the mite *Dermanyssus gallinae* in town flats. Folia Parasitol 18: 364.

Dantzer R (**1986**) Behavioral, physiological and functional aspects of stereotyped behavior: a review and a re-interpretation. J Anim Sci 62: 1776-1786.

Dantzer R, Mormède P (**1983**) Stress in farm animals: a need for reevaluation. J Anim Sci 57: 6-18.

Davis GS, Siopes TD (**1989**) Ontogeny of daily rhythmicity in plasma corticosterone and variation in sensitivity of the corticosterone response in turkey poults. Poultry Sci 68: 423-427.

Haag-Wackemagel D (**2005**) Parasites from feral pigeons as health hazard for humans. An Appl Biol 147: 203-210.

Jungmann R, Ribbeck R, Eisenblätter S, Schematus H (**1970**) Zur Schadwirkung und Bekämpfung des *Dermanyssus-gallinae* und Federlingsbefalls bei Legehennen. Monatsh Veterinarmed 25: 28-32.

Kilpinen O (**2001**) Activation of the poultry red mite, Dermanyssus gallinae (*acari*: *Dermanyssidae*), by increasing temperatures. Exp Appl Acarol 25: 859-867.

Kilpinen O, Roepstorff A, Permin A, Nørgaard-Nielsen G, Lawson LG Simonsen HB (**2005**) Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). British Poult Sci 46: 26-34.

Kirkwood AC (**1967**) Anaemia in poultry infested with the red mite *Dermanyssus gallinae*. Vet Rec 80: 514-516.

Kokot F, Stupnicki R (**1985**) Radioimmunologiczne i radiokompetycyjne metody używane w klinice. 1st ed., PZWL, Warszawa.

Kowalski A, Sokół R (**2006**) Wpływ ptaszyńca *Dermanyssus gallinae* na poziom kortykosteronu oraz wskaźników immunologicznych i hematologicznych u kur niosek. Med Weter 62: 1188-1190.

Ladewig J (**1987**) Endocrine aspects of stress: evaluation of stress reactions in farm animals. In: Wiepkema PR, van Adrichem PWM (eds) Biology of stress in farm animals: an integrative approach. Martinus Nijhoff Publishers, Dodrecht, pp 13-25.

Nisbet AJ, Billingsley PF (**2000**) A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. Int J Parasitol 30: 19-27.

Nordenfors H, Höglund J (**2000**) Long term dynamics of *Dermanyssus gallinae* in relation to mite control measures in aviary systems for layers. British Poult Sci 41: 533-540.

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

Oliverio A (**1987**) Endocrine aspects of stress: central and peripheral mechanisms. In: Wiepkema PR, van Adrichem PWM (eds) Biology of stress in farm animals: an integrative approach. Martinus Nijhoff Publishers, Dodrecht, pp 3-12

Petrov D (**1975**) Study of *Dermanyssus gallinae* as a carrier of *Pasteurella multocida*. Vet Med Nauki 12: 32-36.

Pilarczyk B, Balicka-Ramisz A, Ramisz A, Pająk B (**2004**) Wpływ inwazji *Dermanyssus gallinae* na zdrowotność i produkcyjność kur niosek. Med Weter 60: 874-876.

Prabhakaran V, Chitravel V, Kokilaprabhakaran S, Jayanthy K (**1997**) Heterophil/lymphocyte ratio response in chickens under stress. Indian Vet J 74: 261-262.

Quan BZ, Shao Y, Huang RJ, Chen LJ, Xie DS, Zhang SM, Hua XB (**1995**) Study on the effects of shipping stress on commercial broilers. Experimental observation of some haematological values. Chin J Vet Med 21: 17-20.

Ramsay GW, Mason PC, Hunter AC (**1975**) Chicken mite (*Dermanyssus gallinae*) infesting a dog. New Zealand Vet J 23: 155-156.

Reshetnikov PT (**1967**) The tick *Dermanyssus gallinae* - carrier of fowl spirochaetosis. Veterinariia 44: 48-49.

Rivier C, Rivest S (**1991**) Review. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol Reprod 45: 523--532.

Rosen S, Yeruham I, Braverman Y (**2002**) Dermatitis in humans associated with the mites *Pyemotes tritici*, *Dermanyssus gallinae*, *Ornithonyssus bacoti* and *Androlaelaps casalis* in Israel. Med Vet Entomol 16: 442-444.

Skierska B (**1968**) Przypadek masowego zaatakowania ludzi przez roztocze ptasie *Dermanyssus gallinae* (Redi, 1674) (Garmasoidea, Dermanyssidae). Wiad Parazytol 14: 322--326.

Soon-Il K, Jee-Hwan Y, Jun-Hyung T, Young-Joon A (**2004**) Acaricidal activity of plant essential oils against Dermanyssus gallinae (*Acari: Dermanyssidae*). Vet Parasitol 120: 297-304.

Szafrańska B, Zięcik A, Okrasa S (**2002**) Primary antisera against selected steroids or proteins and secondary antisera against gamma globulins – an available tool for studies of reproductive processes. Reprod Biol 2: 187-204.

Turner CA, Lewis MH, King MA (**2003**) Environmental enrichment: effects on stereotyped behavior and dendritic morphology. Dev Psychobiol 43: 20-27.