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Effect of Dietary Humate and Organic Acid Supplementation on Social Stress Induced by High Stocking Density in Laying Hens

[1]Ebru Cetin, [2]Berrin Kocaoglu Guclu and [1]Nazmi Cetin
[1]Department of Physiology, [2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Erciyes, Kayseri, Turkey

Abstract: This study was conducted to determine the anti-stress effect of the dietary humate and organic acid supplementation on laying hens when subjected to high stocking density as a social stress factor. A total of hundred, 40 weeks, brown laying hens were housed at two different stocking densities of 287.7 (high density) and 500 (low density) cm^2/hen. For the control group, 16 hens were randomly assigned to 4 groups, 4 replicates of 4 birds each and were kept in low density. The control group received a basal diet. The remaining 84 hens were divided into 3 treatment groups, 4 replicates of 7 birds each and were housed at high density. The treatment group were fed either a basal diet (crowded control) or the basal diet supplemented with either 0.15 humate (Humate group) or 0.20 % organic acid (organic acid group) of diet for 60 days. The results show that in hens kept in high density heterophils and Heterophil to Lymphocyte (H:L) ratios, an indicator of stress were raised while lymphocytes decreased. Humate supplementation resulted in significant increases in the lymphocyte counts and significant decreases in the heterophil counts and H:L ratios compared with those of the crowded control. The heterophils, lymphocytes and H:L ratio were not influenced by organic acid treatment. The present results suggest that humate supplementation to diet may be a favorable alternative for help poultry to cope with social stresses.

Key words: Humate, organic acid, social stress, laying hen, lymphocyte, Turkey

INTRODUCTION

Humic Acids (HA) are organic compounds naturally present in water and soil. Humic acid is resulting from decomposition of organic matter, particularly plants and humus contain humic acid, fulvic acid, ulmic acid and some microelements (Stevenson, 1994). Several studies have shown HA having antiinflammatory (Kuhnert *et al*., 1982; Joone and van Rensburg, 2004), immunostimulatory (Pukhova *et al.,* 1987; Joone *et al*., 2003), antimicrobial (Riede *et al*., 1991) and antiviral properties (Lu *et al*., 2002) but whether HA is beneficial to social stress (high stocking density, etc.) is not known. In recent years, it has been reported that addition of humate into layer diets can improve egg production, egg weight and feed efficiency (Yoruk *et al*., 2004; Kucukersan *et al*., 2005; Hayirli *et al*., 2005). Organic acids have been studied as a tool to reduce unwanted bacteria during poultry production (Griggs and Jacob, 2005). Some researchers have suggested that several organic acids can improve growth performance, feed efficiency, mineral absorption and phytate-P utilization when supplemented in nonruminant diets (Boling *et al*., 2000). Exposure of fowls to stress is an inevitable event in poultry husbandry. Fowls are subjected to frequent stress factors such as environmental, nutritional, physical, social, pathological and social stress such as high stocking density and poor body weight uniformity. Previous studies have showed that high cage density decrease body weight (Cunningham and Gvaryahu, 1987; Davami *et al*., 1987) egg production and egg weight (Cunningham and Ostrander, 1982; Quart and Adams, 1982) and increase mortality (Roush *et al*., 1984; Adams and Craig, 1985) and therefore it is important to have an effective management program to minimize harmful effects of stress on the performance and health of the birds. Under the long term stress condition or repeated stress, birds became fatigued and weak. These conditions lead to bird starvation and infectious disease (Freeman, 1987).

Several physiological variables have been assessed as indicator of stress or animal welfare. Chief among these variables are differential leucocyte count, H:L ratio and corticosteron sampling (Gross and Siegel, 1983; Maxwell, 1993). It was reported that corticosterone was not useful measures of long-term stress (Gibson *et al*., 1986; Cunningham *et al*., 1988). The H:L ratio is more

Corresponding Author: Ebru Cetin, Department of Physiology, Faculty of Veterinary Medicine, University of Erciyes, Kocasinan, Kayseri, Turkey

reliable indicator of the long-term stress than corticosteron sampling (Gross and Siegel, 1983). Nutritional modifications can help poultry to cope with stresses. In this connection, several feed compounds such as ascorbic acid, yeast, tryptophan, niacin and vitamin E have been used to reduce stress in poultry (Campo and Davila, 2002).

However, information concerning the effects of humate or organic acid supplementation on blood stress indicators in laying hens exposed to high cage density has not been reported.

Therefore, the aim of this study was to conduct an experiment related to the effects of humate and organic acid supplementation on differential leukocyte counts and H:L ratio in laying hens exposed to high cage density as a social stress factor.

MATERIALS AND METHODS

Animals: A total of hundred, 40 weeks, brown laying hens were housed at different densities of 287.7 (high density) and 500 (low density) cm^2/hen.

Experimental design: For the control group, 16 hens were randomly assigned to 4 groups, 4 replicates of 4 birds each. The control group was fed a basal diet (Table 1) and was kept in low density. The remaining 84 hens were divided into 3 treatment groups, 4 replicates of 7 birds each and were housed at high density. The treatment groups were fed either a basal diet (crowded control group) or the basal diet supplemented with either 0.15 humate (Farmagulator-Dry, Farmavet International Inc., Kocaeli, Turkey) each kilogram of humic acids contained 160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic and humatomelanic acids), 663.3 mg SiO_2 and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; CO, 0.2 mg; I, 1 mg; Se, 0.5 mg and Al, Na, K, Mg and P in trace amounts) (Humate group) or 0.20% organic acid (Sal-Tech, Alltech, Nicholasville, KY, USA; containing propionic acid 15%, formic acid 24% and ammonium hydroxide 3%) (organic acid group) of diet for 60 days. During the experiment, hens were provided with water and feed *ad libitum* and were exposed to a 17 L:7 D lighting schedule.

Sample collection: At the end of the 60 days treatment period, 4 blood samples from each sub-group, totaling 16 samples from each main group were taken for leukocyte profiles and H:L ratio by puncturing the brachial vein. Two blood smears for each animal were prepared.

Table 1: Composition and calculated values of basal (control) diet

Ingredients	Percent	Calculated values	Values
Corn	34.136	ME, Kcal/kg	2750.00
Wheat	30.000	Dry matter (%)	88.85
Full fat soybean	10.000	Crude protein (%)	16.50
Soybean meal	6.034	Crude oil (%)	4.44
Limestone	8.309	Crude ash (%)	12.68
Sunflower meal	7.042	Calcium (%)	3.75
Meat-bone meal	3.727	Available phophorus (%)	0.44
DL-methionine	0.125	Lysine (%)	0.77
Lysine	0.027	Methionine (%)	0.38
Phytase	0.070	Methionine-sistine (%)	0.69
Salt	0.273	Linoleic acid (%)	1.96
Vitamin-mineral pre-mix*	0.257		
Chemical analyses			
Dry matter (%)	88.660		
Crude protein (%)	17.250		
Crude oil (%)	4.400		
Crude ash (%)	11.100		

Values are expressed as percent * per kg of vitamin-mineral pre-mix provides vitamin A 4800 000 IU; vitamin D_3 800, 000 IU; vitamin E 8 g; vitamin K_3 1.2 g; B_1 vitamin 1.2 g; B_2 vitamin 2 g; niacin 8 g; calcium pantothenate 8 g; vitamin B_6 2 g; vitamin B_{12} 0.006 g; folic acid 1.87 g; D-biotin 0.02 g; vitamin C 20 g; choline chloride 60g; canthaxanthin 0.6 g; manganese 32 g; iron 24 g; zinc 24 g; copper 2 g; cobalt 0.08 g; iodine 0.4 g; selenium 0.06 g

All slides were dried for at least 10 min, stained for 5 min in May-Grunwald and for 20 min in Giemsa and then rinsed with distilled water. They were allowed to air dry. Total of 100 leukocytes including heterophils, lymphocytes, monocytes, eosinophils and basophils, were counted on slides and classified using oil immersion microscopy at 100X. The H:L ratios were determined by dividing the number of heterophils by that of lymphocytes. The heterophil and lymphocyte counts and the H:L ratio were used as indicators of stress in this study.

Statistical analysis: Data were analysed using the SPSS for windows software, Version 10.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between groups were determined by Analysis of Variance (ANOVA). When the differences were significant, Tukey HSD test was performed. Mean values were considered significantly different at $p<0.05$.

RESULTS

Supplementation of humate and organic acid to the crowded laying hens' diet on heterophil and lymphocyte counts and H:L ratios are shown in Fig. 1-3. The heterophil percentage and the H:L ratio of hens in the crowded control group was significantly higher ($p<0.05$) than in hens in the standard control group. On the other hand, there was a significant decrease ($p<0.05$) in lymphocyte percentage in the crowded control group compared with the standard control group.

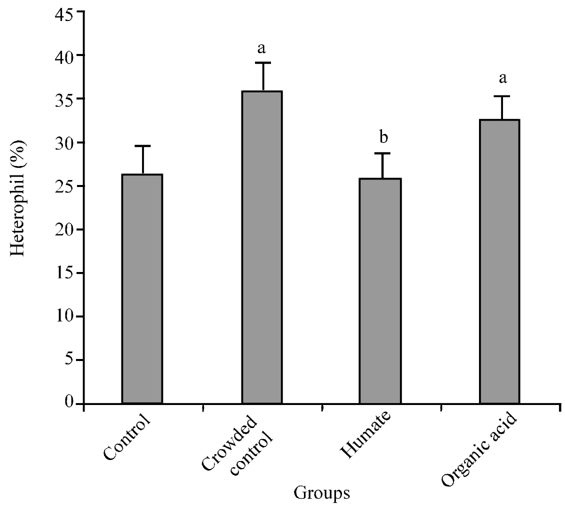

Fig. 1: The heterophil percentage in the control and treatment groups. [a]Significantly different from the control group ($p<0.05$). [b]Significantly different from the crowded control group ($p<0.05$)

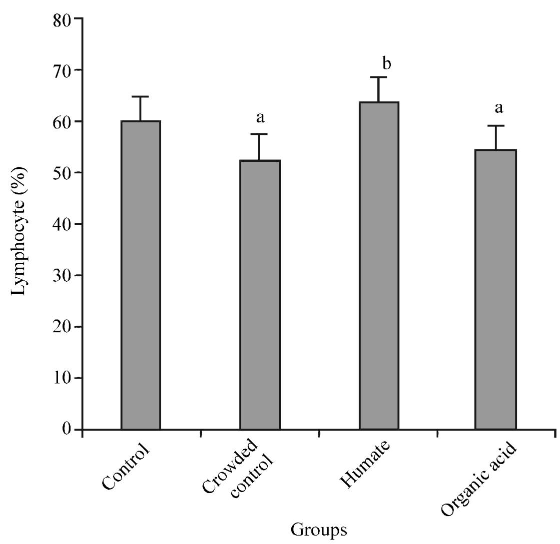

Fig. 2: The lymphocyte percentage in the control and treatment groups. [a]Significantly different from the control group ($p<0.05$). [b]Significantly different from the crowded control group ($p<0.05$)

Addition of humate to the crowded laying hen diets caused a significant ($p<0.05$) increase in the lymphocte count and significant decrease ($p<0.05$) in the heterophil caount and the H:L ratio compared with the crowded control and organic acid supplemented group.

When compared with the crowded control group, heterophil, lymphocyte and basophil counts and H:L ratio were not significantly influenced ($p>0.05$) by dietary supplementation with organic acid.

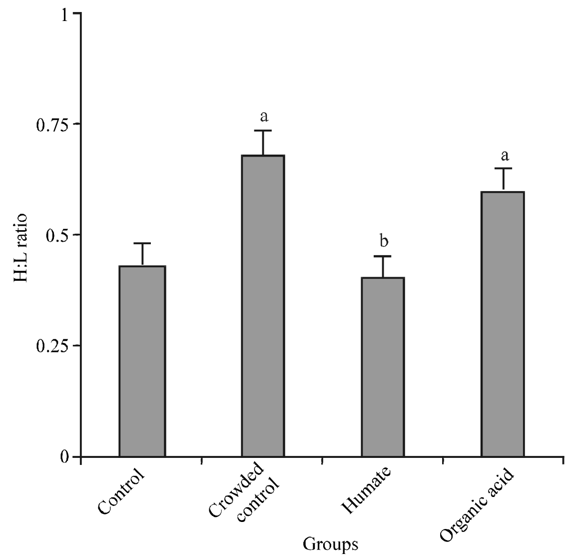

Fig. 3: The H:L ratios in the control and treatment groups. [a]Significantly different from the control group ($p<0.05$). [b]Significantly different from the crowded control group ($p<0.05$)

DISCUSSION

Increasing population density of laying hens is a management practice used for reducing costs regarding housing, labor and equipments. However, increasing stocking density has a negative effect on animal performance and well-being (Davami *et al.*, 1987; Freeman, 1987). Social factors in animals reared at high densities appear to be more important than environmental factors in causing stress (Keeling and Duncan, 1991). Stress imparts physiological responses from the Hypothalamic Pituitary Adrenal (HPA) axis with consequent changes in stress hormone levels such as corticosterone and catecholamines. The corticosteron concentrations and H:L ratio are commonly used as indicators of stress. Increases in plasma corticosteroid concentrations have been reported to be an indicator of short-term stress in poultry (Gross and Siegel, 1983; Craig *et al.*, 1986; Gibson *et al.*, 1986; Cunningham *et al.*, 1988) whereas increases in H:L ratio have been reported to be an indicator of long-term stress (Gross and Siegel, 1983; Siegle, 1985; Maxwell *et al.*, 1992; Maxwell, 1993; McFarlane *et al.*, 1989). In the avian, heterophils and lymphocytes are more responsive to stressor than are the other leukocytes (Maxwell *et al.*, 1992). Therefore in this study, heterophil and lymphocyte counts and the H:L ratio were used as indicators of stress status in hens exposed to high cage density. The results of this study showed that high stocking density produced social stress in hens kept in high cage density as evidenced by the increased heterophil count and heterophil to lymphocyte ratio. Stocking density has been considered as a social stressor in hens (Freeman, 1987; Savory *et al*., 1999; Cheng *et al*., 2003). In the present study, the heterophil to lymphocyte ratio of the crowded group having 7 hens cage^{-1} was higher ($p<0.05$) than those of the standard group having 4 hens cage^{-1}. The higher H:L ratio of the groups having 7 hens cage^{-1} reflect higher stress conditions. As regards the effect of high stocking density on H:L ratio, Onbasilar and Aksoy (2005) reported that H:L ratio of group having 5 hens cage^{-1} was higher than those of groups having 1 or 3 hens cage^{-1}. A study conducted by Hester *et al*. (1996) revealed that hens kept in multiple-bird cages (362 cm^2 bird^{-1}) had higher H:L ratio than those of kept in single-bird cages (1,085 cm^2 bird^{-1}). Similarly, Cravener *et al*. (1992) observed that H:L ratio of birds housed at 900 and 1100 cm^2 bird^{-1} were higher than those of 500 and 700 cm^2 bird^{-1}. The above reports support the findings of the present study. The increase in the heterophil and H:L ratio induced by high stocking density could be related to glucocorticoid-induced change in neutrophil trafficking and release from bone marrow reserves (Davis *et al*., 2008; Weiss and Wardrop, 2010). Similarly, the reduction in lymphocyte ratio of hens kept in high cage density may be related to glucocorticoid-induced alterations in the traffing or redistribution of lymphocytes from the blood to other body compartments (Dhabhar, 2002). This migration of lymphocytes from the main circulation causes a significant decrease in their circulating numbers. In contrast to the findings, several reseachers have reported that increased cage density had no effect on the H:L ratio (Patterson and Siegel, 1998; Turkyilmaz, 2008). This difference may be due to season, housing condition, genotype and age.

The present results revealed that humate supplementation to the crowded laying hens diet decreased the heterophil percentage and the H:L ratio while increased lymphocyte percentage as compared to the crowded control group. This means that humate supplementation minimized stress in the laying hens exposed to high stocking density. No research regards as the effect of humate on these hematological parameters in laying hens exposed to chronic stress has been reported in the literature. In a previous research, Rath observed that humate supplementation caused a decrease in heterophil count and heterophil to lymphocyte ratio in non-stressed broiler chickens. Relating to the mechanism of humic substances on lymphocyte response, it has been shown that a water-soluble humate called oxihumate increased the proliferative response of lymphocytes via the increased production of IL-2 and the expression of IL-2 receptors on lymphocye which resulted in the enhancement of the activity of IL-2 producing cells (Joone *et al*., 2003). It is also thought that humate reduces the production of stress causing hormones (Islam *et al*., 2005).

In the present study, researchers observed that heterophils, lymphocytes and H:L ratios of hens exposed to high stocking density were not influenced by the organic acid treatment. On the other hand, Tollba *et al*. (2010) reported that addition of aromatic herbal extract and blended with organic acids for 12 weeks to chicks diet under the cold stress condition increased heterophil and lymphocyte counts. Hassan *et al*. (2009) suggested that acetic acid supplementation may have played a role in ameliorating the heat stress-induced changes in H:L ratio of broiler chicks. This difference can be attributed to study population (e.g., in age and breed), composition of diet and dose of given organic acid.

CONCLUSION

The present study provides the 1st evidence that humate treatment has a strong anti-stress effect against social stress. The present results suggest that humate supplementation to diet may be a favorable alternative way for help poultry to cope with chronic stresses and minimize harmful effects of chronic stress on the performance and health of the hens.

It is also reported that stress may cause immunosuppression and leads to infectious disease in poultry (Livestock transport). In this respect, it may be assumed that humate treatment may improve immune response in hens housed at high density by reducing stress level. The potential protective effect of humate in the prevention of chronic stress would be investigated in further studies.

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