

Nutrition Practice to Alleviate the Adverse Effects of Stress on Laying Performance, Metabolic Profile, and Egg Quality in Peak Producing Hens: I. The Humate Supplementation

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ABSTRACT : This experiment was conducted to determine the effects of cage density (CD) and humate supplementation (HS) on laying performance, metabolic profile, and egg quality during the peak production period in hens. Lohman layers (n = 180, 46 wks of age) were blocked according to the location of cages and then allocated randomly to two levels of CD (4 or 6 hens per cage or 540 vs. 360 cm²/hen) and three levels of HS (0, 0.15, and 0.30%). Egg production (EP) and feed consumption (FC) were measured daily; egg weight was measured bi-weekly; and BW was measured before and after the experiment. Blood and additional egg samples were obtained at the end of the experiment for determination of metabolic profile and egg quality. The data were analyzed using two-way ANOVA as repeated measures. Except for FC, CD did not affect laying performance parameters. Hens placed in high-density cages had lower FC than hens placed in normal-density cages. Increasing HS level linearly increased FC, EP, and feed conversion ratio (FCR). There was a CD by HS interaction effect on FC and EP. Hens placed in high-density cages had greater serum glucose, total protein, albumin, globulin, Ca, and P concentrations and tended to have greater serum corticosterone concentration than hens placed in normal-density cages. Increasing HS level linearly increased serum glucose, total protein, albumin, globulin, creatine, and Ca concentrations and linearly decreased serum triglyceride and very low-density lipoprotein concentrations. There was a CD by HS interaction effect on serum glucose and albumin concentrations. There were no alterations in egg quality parameters in response to increasing CD. Albumen index and Haugh unit decreased linearly and other egg quality parameters did not change as HS level increased. In conclusion, increased caging density adversely affected metabolic profile, despite insignificantly deteriorating laying performance. Moreover, benefits from humate supplementation seem to be more noteworthy for hens housed in stressing conditions than for hens housed in standard conditions. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 9 : 1310-1319)

Key Words : Cage Density, Humate, Laying Performance, Metabolic Profile, Egg Quality, Peak-producing Hens

INTRODUCTION

Increasing caging density was used to become a common approach in commercial egg production in order to decrease investment cost and increase profit per hen (Koelkebeck and Cain, 1984). Nowadays, animal health and welfare in relationship to caging density are popular issues due to physical and psychological stress (Odendaal, 1994; Barnett and Hemsworth, 2003; Miao et al., 2005). Although it is postulated that decreases in laying performance and poor welfare are more directly related to other housing conditions (i.e., temperature, humidity, and air quality) than increased caging density per se (Dawkins et al., 2004), these adverse effects were shown to depend on the degree of

caging density in numerous research articles (Hughes, 1975; Craig et al., 1986; Havenstein et al., 1989). Thus, the adverse effects on performance parameters are linked directly and/or indirectly to other constraints associated with increasing cage density (Hughes, 1975). Limited feeder space due to increased caging density may compromise feed consumption, egg production, and egg weight (Robinson, 1979; Cunningham and Ostrander, 1981). Discomforting conditions due to crowding also may depress health status and immune function as reflected by decreased percentages of heterophil and leukocyte, their ratio, superoxide production by neutrophils, and phagocytic activity by neutrophils (Tsukamoto et al., 1994).

Because National Research Council (NRC, 1994) assessed nutrient recommendations based on low density, hens housed at increased caging densities may require greater density of nutrients or additional supplements. In response to increasing dietary protein concentration, partial alleviations in depressed laying performance in hens placed in high-density cages were shown to be inconsistent (Bell et al., 1983; Moran, 1986). Moreover, increasing lysine level from 0.675 to 0.775% with 0.05% increments did not alleviate the adverse effects of increased caging density (344, 516, and 1,032 cm²/hen) (Brake and Peebles, 1992).

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To improve health status of hens, Dafwang et al. (1987) supplemented broiler chicks reared under high stocking density (from 0.047 to 0.019 m²/chick) with subtherapeutic antibiotic. High density caused reduction in weights of the bursa and thymus and antibiotic supplementation failed to ameliorate these reductions. Probiotic supplementation also failed to ameliorate depressed laying performance parameters due to increased caging density (Davis and Anderson, 2002).

Humates are a part of fertilizers and derived from decomposed plant matter by bacteria. Active ingredients of humates consist of humus, humic acid, fulvic acid, ulmic acid, and some microelements (Senn and Kingman, 1973). Recognition of humates as feed additive in animal production is very new. Earlier time, humates were used as a part of replacement therapy for malnutrition, diarrhea, and increased feed conversion efficiency in pseudoruminants and carnivores (Kühnert et al., 1989, 1991). The safety level of humates ranges from 3 to 5% in broilers (Stepchenko et al., 1991), antitoxic (Stackhouse and Benson, 1989), antiinflammatory (Joone and van Rensburg, 2004), and immunostimulatory (Joone et al., 2003) properties of humates are widely studied for human and veterinary medicine to enhance immune potency and health status. Humates were shown to influence protein partitioning and enhance muscle growth in broilers (Zhorina and Stepchenko, 1991). Remarkable alleviations in electrolyte balance and enhancements in immune potency of ruminants (Griban et al., 1991) and turkeys (Parks et al., 1986) and improvements in laying performance parameters in hens (Yoruk et al., 2004) in response to humate supplementation were also reported. It was hypothesized that increasing caging density would depress laying performance and metabolic profile and deteriorate egg quality and humate supplementation would improve these parameters. Moreover, due to biopotent effects of humate, its supplementation would benefit more to hens that are exposed to stressing condition. The objective of this experiment therefore was to evaluate the effects of cage density and humate supplementation on laying performance, metabolic profile, and egg quality during the peak production period.

MATERIALS AND METHODS

Animal, treatment, and management

The Research Animal Ethic Committee of Atatürk University permitted to conduct this experiment. One hundred and eighty Lohman layers, 46 wks of age with uniformity of 94% (the number of hens weighing between 0.9-1.1% of the mean BW), were blocked according to the location of cages (48×45×45 cm, width×depth×height). After two weeks of the adaptation period, hens were

assigned randomly to two levels of caging density (4 vs. 6 hens per cage providing 12 vs. 8 cm feeder space per hen or 540 vs. 360 cm²/hen) and three levels of humate (Farmagülator DRY™ Humate, Farnavet International Inc., Kocaeli 41400, Turkey) supplementation (0, 0.15, or 0.30%) from wk 48 to 58. Each group was replicated in 6 cages. The experimental diets were formulated to be isocaloric and isonitrogenous and meet the NRC nutrient requirements (NRC, 1994). The basal diet consisted of 46.00% corn, 21.00% soybean meal, 7.00% wheat, 3% barley, 8.75% wheat bran, 2.00% molasses, 9.00% limestone, 0.40% salt, 2.00% dicalcium phosphate [Each kilogram contained Ca, 24% and P, 17.5%], 0.40% vitamin-mineral premix [Each kilogram contained vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU; DL- α -tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamin, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B₁₂, 15 μ g; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg], 0.15% ethoxyquin as an antioxidant, 0.15% methionine as DL-methionine, 0.15% lysine as L-lysine hydrochloride. The basal diet contained average of 89.0% DM and 2,690 kcal/kg energy, 16.7% CP, 3.6% crude fiber, 3.16% ether extract, 10.4% ash, 2.65% Ca, and 0.71% P on as fed-basis. In the experimental groups, humate [Each kilogram contained polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids), 160 mg; SiO₂, 663.3 mg; 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)] was included to the basal diet at expense of wheat bran. During the experimental period, hens were fed *ad libitum* once daily at 08:30 hr and water nipples were available all the times. Hen house was lit for 17 h.

Sample collection and analytical procedure

Feed samples were analyzed for DM, CP, crude fiber, ether extract, and ash contents (AOAC, 1990). Metabolizable energy, Ca, and P contents of the experimental diets were calculated from tabular values of feedstuffs (NRC, 1994). Feed consumption and egg production were recorded daily; egg weight was measured bi-weekly; and body weights were measured at the beginning and the end of the experiment. Feed conversion ratio was expressed as kilogram of feed consumed per kilogram of egg produced.

To determine metabolic profile, about 3 ml of blood samples were drawn from wing vein of two hens from each cage into additive-free vacutainers at the end of the experimental period. Serum was obtained following centrifugation at 3,000 g for 15 min at 20°C. Aliquots were kept at -20°C until laboratory analyses for glucose, triglyceride, cholesterol, very low-density lipoprotein, total

Table 1. The effects of cage density and humate supplementation level on laying performance parameters during the during the peak production period in hens

Treatments		Response variables ¹							
Cage density (n) ²	Humate level (%)	Feed consumption (g/d)	Egg production (%)	Cracked egg yield (%)	Egg weight (g)	Initial BW (kg)	Final BW (kg)	Relative BW change (%)	FCR (kg feed/kg egg)
4 hens per cage	0	126.4	88.4	0.56	68.1	1.68	1.67	0.52	1.94
	0.15	126.0	91.8	0.13	65.6	1.62	1.67	-3.09	1.93
	0.30	128.9	92.0	0.14	67.5	1.68	1.71	-1.92	1.96
6 hens per cage	0	121.5	90.7	0.64	65.5	1.70	1.67	1.42	1.89
	0.15	123.4	89.4	0.53	66.2	1.59	1.62	-1.73	1.95
	0.30	130.0	92.3	0.44	65.6	1.69	1.64	2.50	2.00
SEM		1.2	0.8	0.18	1.8	0.04	0.03	1.76	0.02
ANOVA		p > F							
Cage density (CD)		0.04	0.89	0.09	0.39	0.91	0.12	0.13	0.87
Humate level (HL)		0.0001	0.003	0.19	0.88	0.11	0.52	0.15	0.008
Linear effect		0.0001	0.001	0.10	0.90	0.91	0.89	0.70	0.002
Quadratic effect		0.06	0.75	0.48	0.63	0.12	0.26	0.06	0.72
CD×HL		0.05	0.008	0.67	0.67	0.86	0.48	0.56	0.16
Time (T)		0.0001	0.0001	0.45	0.12	-	-	-	0.0001
CD×T		0.0001	0.19	0.75	0.27	-	-	-	0.0001
HL×T		0.47	0.83	0.61	0.43	-	-	-	0.66
CD×HL×T		0.004	0.97	0.51	0.51	-	-	-	0.01

¹ Relative BW change = (initial BW - final BW)×100/initial BW; FCR = feed conversion ratio (kg feed consumed per kg egg produced).

² 540 vs. 360 cm²/hen.

protein, albumin, globulin, creatine, Ca, and P using commercial kits (DDS[®], Diasis Diagnostic Systems Co., Istanbul 80270, Turkey) as well as corticosterone using RIA (Beuving and Vonder, 1977). Intraassay CV was 12.8% in hormone analysis.

To assess egg quality parameters, another sample of three eggs was randomly collected from each cage at the end of the experimental period. Egg quality parameters were calculated using following formulas and methods as summarized by Ergün et al. (1987): Shape index (%) = (egg width, cm/egg length, cm)×100; shell strength (kg/cm²) was determined by using machine with the "spiral pressure system"; shell thickness (mm×10⁻²) was determined in 3 different parts by using "micrometer"; albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm)×100; yolk index (%) = (yolk height, mm/yolk diameter, mm)×100; yolk color was determined by using commercially available "yolk color fan" according to the CIE standard colorimetric system (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit = 100×log (AH+7.57-1.7×EW^{0.37}), where AH = albumen height, mm and EW = egg weight, g.

Statistics

Factorial arrangements of two caging densities and three humate supplementation levels were tested in a complete randomized block design experiment. The cage location was considered as a blocking factor to eliminate the

confounding effect of possibility of differences in air-flow and light-intensity. Body weight measured before the experiment was used as covariate for statistical analyses of all response variables. Two-way ANOVA was then conducted using the Mixed Procedure (SAS, 1998) as repeated measures with first-order autoregressive covariance structure as time being subplot. The linear model to test the effects of treatments on laying performance parameters was as follows:

$$Y_{ijkl} = \mu + b_0 + b_1(\text{Cov}_{\text{BW}}) + B_i + CD_j + HL_k + (CD \times HL)_{jk} + \text{Error A} + T_l + (CD \times T)_{jl} + (HL \times T)_{kl} + (CD \times HL \times T)_{jkl} + \text{Error B}$$

where, Y_{ijkl} = response variable, μ = population mean, b_0 = intercept, b_1 = slope, Cov_{BW} = covariate (BW = body weight), B_i = block ($i = 1^{\text{st}}$ cage at lower level by aisle to 6^{th} cage at upper level by window), CD_j = cage density ($j = 4$ or 6 hens per cage), HL_k = humate level ($k = 0$ to 0.3%), $(CD \times HL)_{jk}$ = cage density j and humate level k interaction, Error A = whole plot error, T_l = time ($l = \text{wk}$ relative to initiation of the experiment), $(CD \times T)_{jl}$ = cage density j and time l interaction, $(HL \times T)_{kl}$ = humate level k and time l interaction, $(CD \times HL \times T)_{jkl}$ = cage density j and humate level k , and time l interaction, and Error B = subplot error. Because blood and egg were sampled only at the end of the experimental period, time effect and its possible interaction terms were omitted from the linear models for statistical

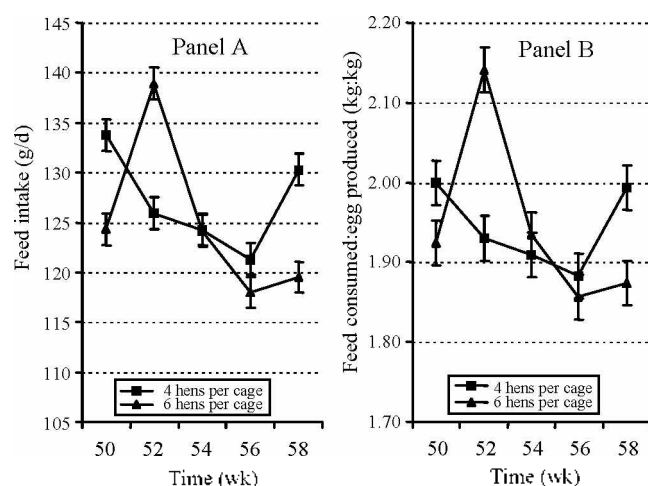


Figure 1. The effects of cage density by time interaction on feed intake (SEM = 1.6 and $p < 0.05$) [Panel A] and feed conversion ratio (SEM = 0.03 and $p < 0.008$) [Panel B].

analyses of metabolic profile and egg quality parameters. The effects of treatments on response variables were considered to be significant at $p \leq 0.05$ and trend between $0.05 < p \leq 0.10$.

RESULTS

Laying performance

Table 1 shows the effects of caging density and humate supplementation level on laying performance. Except for feed consumption, there was no effect of caging density on other laying performance parameters. Hens placed in high-density cages consumed less amount of feed than hens placed in normal-density cages (125.0 vs. 127.1 g/d, $p < 0.04$). Also, percentage of defective egg tended to be greater for hens placed in high-density cages than for hens placed in normal-density cages (0.53 vs. 0.28%, $p < 0.09$). Humate supplementation did not affect BW and egg weight. Feed consumption ($p < 0.0001$), hen-day egg production ($p < 0.0001$), and FCR ($p < 0.002$) increased linearly; cracked egg yield tended to decrease linearly ($p < 0.10$); and relative body weight change at the end of the experiment tended to decrease quadratically ($p < 0.06$), as humate supplementation level increased. The mean values were 123.9, 124.7 and 129.5 g/d for feed consumption; 89.5, 90.6 and 92.1% for hen-day egg production; 0.60, 0.33, and 0.29% for cracked egg yield; 1.0, -2.3, and 0.3% for relative BW change; and 1.91, 1.94, and 1.98 for FCR in hens supplemented with 0, 0.15, and 0.30% humate, respectively. The magnitude of linear increase in feed consumption in response to increasing level of humate supplementation was also more pronounceable for hens placed in high-density cages than for hens placed in normal-density cages ($p < 0.05$, Table 1). As humate supplementation increased, increase in hen-day

egg production was in a linear fashion for hens placed in normal-density cages, whereas that was in quadratic fashion for hens placed in high-density cages ($p < 0.008$, Table 1).

There were variations in feed consumption, hen-day egg production, and FCR ($p < 0.0001$ for all) as the experiment continued. The mean values were 129.0, 132.4, 124.2, 119.6, and 124.9 g/d for feed consumption; 85.7, 91.0, 92.2, 92.4, and 92.6% for hen-day egg production; and 1.96, 2.04, 1.92, 1.87, and 1.93 for FCR on wk 50, 52, 54, 56, and 58, respectively. Caging density, but not humate supplementation level affected changes in feed consumption ($p < 0.0001$; Figure 1 -Panel A) and FCR ($p < 0.0001$; Figure 1 -Panel B) as the experiment progressed. Feed consumption and FCR for hens placed in normal-density cages density tended to not change, whereas those for hens placed in high-density cages changed as the experiment continued. Moreover, there were also three-way interaction effect of caging density, humate supplementation level and time on feed consumption ($p < 0.004$; Figure 2 -Panel A) and FCR ($p < 0.01$; Figure 2 -Panel B). Increasing level of humate supplementation first decreased and then increased feed consumption and FCR for hens placed in normal-density cages, whereas it first increased and then decreased these parameters for hens placed in high-density cages as the experiment continued.

Metabolic profile

Table 2 summarizes the effects of caging density and humate supplementation level on metabolic profile. Hens placed in high-density cages had greater serum glucose (277 vs. 268, $p < 0.03$), total protein (7.20 vs. 6.38, $p < 0.002$), albumin (2.22 vs. 1.97, $p < 0.0001$), globulin (4.98 vs. 4.41, $p < 0.0009$), Ca (16.3 vs. 16.0, $p < 0.05$), and P (6.92 vs. 5.82, $p < 0.02$) concentrations (all in mg/dL) and tended to have greater serum corticosterone concentration (12.0 vs. 10.8 $\mu\text{g/dL}$, $p < 0.08$) than hens placed in normal-density cages. Increasing level of humate supplementation linearly increased serum glucose ($p < 0.0001$), total protein ($p < 0.001$), albumin ($p < 0.001$), globulin ($p < 0.002$), creatine ($p < 0.008$), and Ca ($p < 0.05$) concentrations and linearly decreased serum TG ($p < 0.02$) and VLDL ($p < 0.03$) concentrations. The mean serum concentrations (mg/dL) were 260, 267, and 290 for glucose; 824, 812, and 809 for TG; 165, 162, and 162 for VLDL; 6.28, 6.72, and 7.38 for total protein; 1.98, 2.06, and 2.26 for albumin; 4.30, 4.67, and 5.12 for globulin; 0.40, 0.45, and 0.50 for creatine; and 16.0, 16.2, and 16.4 for Ca concentrations in hens supplemented with 0, 0.15, and 0.30% humate respectively. Moreover, increasing level of humate supplementation linearly increased serum glucose ($p < 0.0001$; Table 2) and albumin ($p < 0.03$; Table 2) concentrations for hens placed in normal-density cages, whereas it quadratically increased these parameters for hens placed in high-density cages.

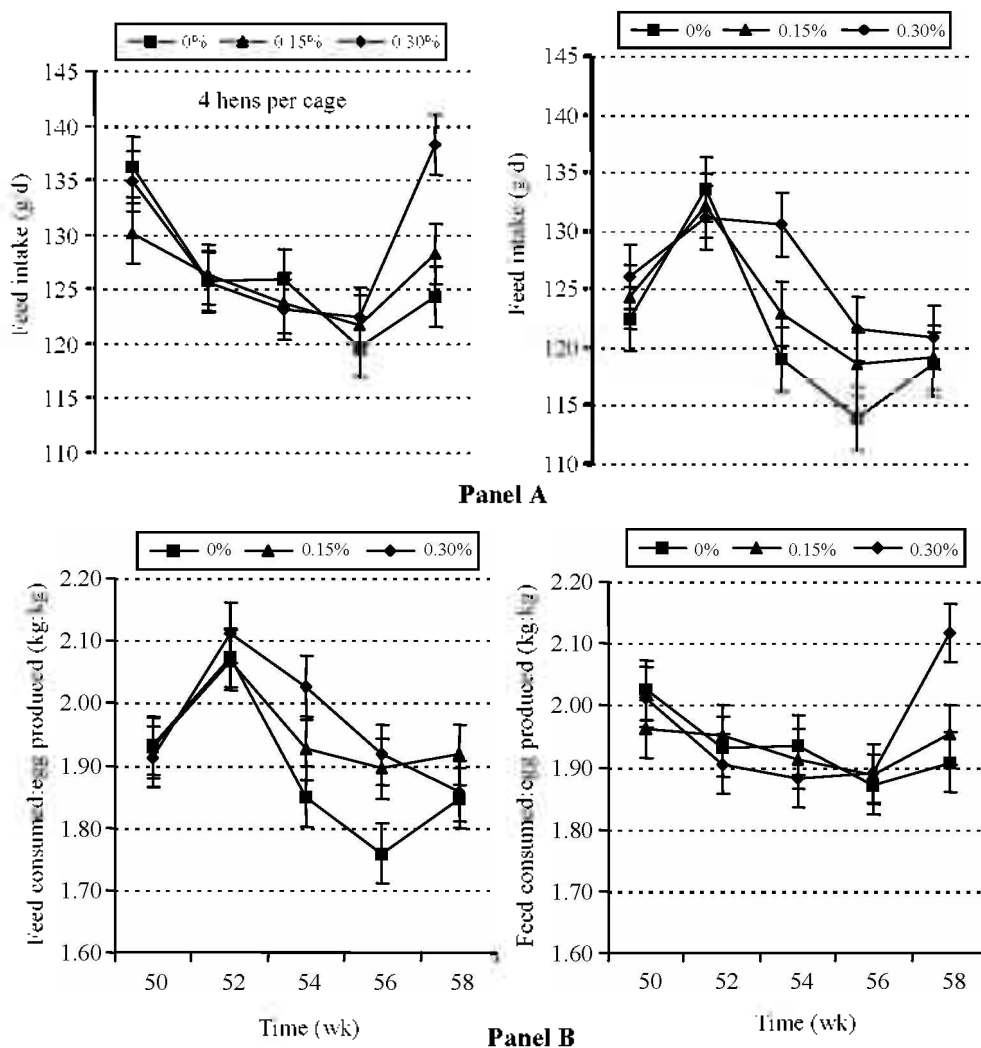


Figure 2. The effect of three-way interaction of cage density, humate supplementation level, and time on feed intake (SEM = 2.7 and $p < 0.004$) [Panel A] and feed conversion ratio (SEM = 0.05 and $p < 0.01$) [Panel B].

Egg quality

Caging density did not affect egg quality parameters (Table 3). Except for increases in albumen index and Haugh unit, there were also no changes in other egg quality parameters in response to humate supplementation (Table 3). Albumen index ($p < 0.01$) and Haugh unit ($p < 0.008$) decreased linearly as humate supplementation level increased and both were highly correlated ($r = 0.94$, $p < 0.0001$). The mean values were 8.94, 7.64, and 7.51% for albumen index and 85.6, 78.5, and 77.8 for Haugh unit for hens supplemented with 0, 0.15, and 0.30% humate, respectively. Interestingly, yolk index for hens placed in normal-density cages increased linearly, whereas that for hens placed in high-density cages decreased linearly with increasing level of humate supplementation ($p < 0.006$; Table 3).

DISCUSSION

Caging density in this experiment was confounded with

feeder space per hen. However, it was intended to create a stressing condition. Numerous studies have performed to evaluate the effects of a great range of stocking densities on laying performance and their results are conflicting. In the present study, no death or aggressive behavior (vent pecking, nervousness, and fearfulness, etc.) observed due to increased caging density. Except for depression in feed consumption and increase in cracked egg percentage, increasing caging density did not influence other laying performance parameters (Table 1). At the densities of 450, 525, 600 and 750 cm² area per hen, Bishop (2004) reported no changes in laying performance parameters. In the literature, the effects of caging density are highly variable and seem to depend on the degree of crowding. In general, the adverse effects of increased caging density on laying performance parameters could be related to restraining physical activity and disturbed social behaviors (Hughes, 1975; Cunningham, 1988; Okpokho et al., 1987). Depression in feed consumption in this experiment could be attributed to reduced feeder space (Nicol, 1987) or

Table 2. The effects of cage density and humate supplementation level on metabolic profile during the during the peak production period in hens

Treatments		Response variables ¹										
Cage density (n) ²	Humate level (%)	Cort	Glc	TG	Chol	VLDL	TP	Alb	Glo	Cre	Ca	P
4 hens per cage	0	10.3	239.3	827.7	159.5	165.5	5.60	1.73	3.87	0.38	15.6	5.43
	0.15	11.1	268.8	802.3	218.0	160.3	6.50	1.98	4.52	0.43	16.2	5.98
	0.30	11.5	294.5	806.3	182.5	161.5	7.05	2.20	4.85	0.50	16.2	6.05
6 hens per cage	0	12.5	280.5	819.3	203.8	163.8	6.95	2.22	4.73	0.42	16.3	7.10
	0.15	11.8	264.8	820.8	181.2	164.0	6.95	2.13	4.82	0.47	16.1	5.75
	0.30	11.6	285.5	811.5	218.7	162.3	7.70	2.32	5.38	0.50	16.5	7.90
SEM		0.6	5.0	6.0	23.2	1.2	0.30	0.07	0.25	0.04	0.2	0.53
ANOVA		p > F										
Cage density (CD)		0.08	0.03	0.31	0.44	0.35	0.002	0.0001	0.009	0.45	0.05	0.02
Humate level (HL)		0.67	0.0001	0.05	0.66	0.06	0.003	0.002	0.009	0.03	0.10	0.12
Linear effect		0.67	0.0001	0.02	0.42	0.03	0.001	0.001	0.002	0.008	0.05	0.19
Quadratic effect		0.44	0.07	0.38	0.68	0.30	0.70	0.37	0.85	0.91	0.95	0.11
CD×HL		0.49	0.0001	0.11	0.17	0.11	0.29	0.03	0.52	0.86	0.12	0.11

¹ Cort = Corticosterone; Glc = Glucose; TG = Triglyceride; Chol = Cholesterol; VLDL = Very low-density lipoprotein.

TP = Total protein; Alb = Albumin; Glo = Globulin; Cre = Creatine. All are in mg/dL, except for corticosterone ($\mu\text{g/dL}$).

² 540 vs. 360 cm²/hen.

increased release of heat via radiation (Robinson, 1979). The number of studies reported that increased caging density decreased feed consumption (Ramos et al., 1986; Cunningham and Gvoryahu, 1987; Brake and Peebles, 1992), hen-day egg production (Roush et al., 1984; Ramos et al., 1986; Cunningham and Gvoryahu, 1987; Cunningham, 1988; Havenstein et al., 1989; Brake and Peebles, 1992; Lee and Moss, 1995a; Sohail et al., 2001), BW (Ramos et al., 1986; Cunningham and Gvoryahu, 1987), and egg weight (Robinson, 1979; Sohail et al., 2001) and increased FCR (Roush et al., 1984; Brake and Peebles, 1992; Lee and Moss, 1995a) and mortality (Roush et al., 1984; Craig and Milliken, 1989; Havenstein et al., 1989; Moinard et al., 1998). In these studies, caging density ranged from 1.394 to 310 cm² area per hen. General industry standard of caging density is 450 cm² per hen and the adverse effects appear to become significant in area per hen less than 697 cm² (Mench et al., 1986). In this experiment, increase in cracked egg yield caused by increased caging density could be related to the physical effect of crowding (Carey et al., 1995), possibly not to alteration in Ca metabolism because serum Ca concentrations were not correlated with outer eggshell quality parameters. However, Hester and Wilson (1986) housed hens at varying degree of densities (from 344 to 1,031 cm²/hen) and reported a linear decrease in hard-shelled egg yield.

To enhance nutrient utilization, improve feed conversion efficiency, and maintain health status, inclusion of humate into rations could be favorable due to primarily its lack of harmful effects on consumers (Onifade et al., 1999). However, studies regarding supplemental humate as a feed additive are limited. Thus, little is known about

mechanism by which humate supplementation enhances well-being and consequently, improves performance in poultry production. Polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids) in humate may have antagonist effects and form chelate to stabilize toxic metals. Moreover, these trace minerals may act as co-factors, and consequently, increase the activity of several enzymes for digestion and utilization of nutrients. In response to humate supplementation, alleviation of the adverse effect of lethal dose of irradiation (Pukhova et al., 1987) and inhibition of formation of N-methyl-N-nitrosourea, a mutagenic compound (Badaev et al., 1989) in rats and decreased deposition of Cr in liver and kidney of fish (Stackhouse and Benson, 1989) and of Cd in chickens (Herzig et al., 1994) after loading may suggest that humate supplementation may benefit to well-being under stressing conditions (Figure 1) and consequently, affect laying performance positively (Table 1 and Figure 2). In this study, positive effects could be related to trace elements having antioxidant role present in humate (Lim and Paik, 2003). Moreover, in agreement with the current study (Table 1), the limited number of studies consistently shows that humates promote growth and increase egg production by altering partitioning of nutrient metabolism (Parks et al., 1986; Stepchenko et al., 1991; Zhorina and Stepchenko, 1991; Yoruk et al., 2004) and reducing mortality (Eren et al., 2000; Yoruk et al., 2004) and improve feed conversion efficiency (Shermer et al., 1998; Eren et al., 2000; Kocabağlı et al., 2002; Yoruk et al., 2004).

Despite of the lack of response in this study, depressions in laying performance parameters could be linked to stress that is associated with agonistic activity, fearfulness, and nervousness due to increased caging density (Okpokho et

Table 3. The effects of cage density and humate supplementation level on egg quality parameters during the during the peak production period in hens

Treatments		Response variables						
Cage density (n) ²	Humate level (%)	Shape index (%)	Shell strength (kg/cm ²)	Shell thickness (mm×10 ⁻²)	Yolk color	Yolk index (%)	Albumen index (%)	Haugh unit
4 hens per cage	0	72.6	1.01	1.09	9.33	39.2	8.88	84.3
	0.15	73.7	1.09	1.06	9.67	39.6	7.25	77.2
	0.30	74.5	1.52	1.12	9.50	42.5	7.67	77.4
6 hens per cage	0	74.2	1.12	1.05	10.00	41.4	9.00	86.8
	0.15	72.8	1.19	1.08	9.83	39.9	8.03	79.8
	0.30	74.6	1.06	1.07	9.33	38.6	7.35	78.2
SEM		1.0	0.22	0.03	0.41	0.9	0.52	2.7
ANOVA		p > F						
Cage density (CD)		0.76	0.64	0.36	0.51	0.58	0.65	0.38
Humate level (HL)		0.34	0.59	0.67	0.70	0.66	0.02	0.01
Linear effect		0.24	0.31	0.44	0.55	0.74	0.01	0.008
Quadratic effect		0.38	0.84	0.66	0.56	0.40	0.21	0.18
CD×HL		0.44	0.35	0.58	0.60	0.006	0.57	0.94

² 540 vs. 360 cm²/hen.

al., 1987; Cunningham, 1988; El-Lethey et al., 2003). However, other studies indicate that increased caging density effect on physically limiting social behavior is controversial and depends on the degree of population size and stocking density (Cunningham et al., 1988; Lee and Moss, 1995b; Carmichael et al., 1999; Anderson et al., 2004). Several researchers worked on metabolic profile to assess indicator of stress. Corticosterone is the major steroid hormone released by the avian adrenal gland (Harvey et al., 1986) and regulates the tissue level of phenyletanolamin-N-methyltransferase, one of the enzymes necessary for the biosynthesis of adrenaline (Culbert and Wells, 1975). Depending upon the level of stocking density, the number of heterophils and blood glucose and corticoid concentrations increase; the number of lymphocytes decreases; and humoral and cell mediated immunity are impaired (Saleh and Jaksch, 1977; Mashaly, et al. 1984; El-Lethey et al., 2003). Other studies, however, reported no changes in serum corticosterone concentration (Koelkebeck and Cain, 1984; Bishop, 2004), the heterophil to lymphocyte ratio, and hemagglutinin titers to sheep erythrocyte antigen in hens housed either at 361 or 482 cm² per bird from wk 20 to 68 (Davis et al., 2000) and those housed at either 1,394 or 697 cm² per hen (Mench et al., 1986), lymphoid organ weight and lymphocyte blastogenesis in broilers (Heckert et al., 2002). The ratio of heterophils to lymphocytes is relatively more stable and considered to be sensitive criterions of stress (Gross and Siegel, 1983; Craig et al., 1986; Bishop, 2004). Despite a trend in increase in serum corticosterone concentration, significant elevations in serum glucose, total protein, albumin, globulin, Ca, and P concentrations may reflect catabolism related to discomforting conditions (Table 2). However, metabolic parameters in this study were in the

physiological range of those measured under normal conditions (Meluzzi et al., 1992). The effects of caging density on bone strength and blood Ca concentration are inconclusive (Moinard et al., 1998). In disagreement with the present results, other studies reported elevation in serum triglyceride (Jensen et al., 1976) and decrease in serum cholesterol (Bishop, 2004) concentrations in hens housed at increased caging densities. Changes in metabolic profile due to humate supplementation may be related to alteration in partitioning of nutrient metabolism and possibly enhancement of immune status, as mentioned above. Moreover, cage density by humate supplementation interaction effect on serum glucose and albumin concentrations may suggest an interruption of proceeding of the catabolic activities due to increased cage density (Table 2).

Similar to the present experiment, other studies also reported no changes in egg quality parameters in response to increased caging density (Mather and Gleaves, 1970; Davami et al., 1987; Carey and Kuo, 1995). Similar to results reported by Bishop (2004), Altan et al. (2002) reported that except for a linear increase in Haugh unit, other egg quality parameters did not change in hens housed at 640, 480, and 384 cm² area per hen. Unlike the present study, Mench et al. (1986) reported that reducing area from 1,394 to 697 cm² per hen caused reduction in shell strength and Hester and Wilson (1986) reported that reducing area from 1,031 to 344 cm² per hen resulted in a linear decrease in hard-shelled egg yield. Although caging density tended to increase percentage of defective eggs, serum calcium concentration was not correlated with outer eggshell quality (eggshell thickness and shell stiffness), which did not differ by increased caging density. Alike our previous experiment (Yoruk et al., 2004), only albumen index and Haugh unit

were affected by humate supplementation. These interrelated parameters are indicators for freshness and grading and do not change by dietary regimen (Silversides and Scott, 2001). Alteration in yolk index in this present experiment is inconclusive (Table 3).

CONCLUSIONS

In this study, the effects of cage density and humate supplementation on laying performance, metabolic profile, and egg quality parameters during the peak production period were evaluated. Increasing caging density decreased feed consumption and did not alter hen-day egg production, BW, egg weight, and feed conversion ratio. These laying performance parameters were improved linearly with increasing humate supplementation up to 0.30%. Increases in metabolic parameters (serum glucose, total protein, albumin, Ca, and P) could be related to elevated catabolic hormones associated with increasing caging density. Moreover, elevated serum corticosterone concentration for hens placed high-density cages could be attributed to stress. Humate supplementation alleviated the adverse effects of increased caging density on hen-day egg production, FCR, and serum glucose and albumin concentrations. Except for the percentage of cracked eggs, albumen index, and Haugh unit, changes in other egg quality parameters were not significant in response to the treatments. In conclusion, humate supplementation improved laying performance, metabolic profile, and inner egg quality parameters. These benefits were more notable for hens housed in stressing condition than for hens housed in standard condition.

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