

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

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**ABSTRACT :** In this experiment, the effects of cage density (CD) and probiotic supplementation (PS) on laying performance, metabolic profile, and egg quality in peak-producing hens were evaluated. After blocking according to the cage location, Lohman layers (n = 180, 46 wks of age) were allocated randomly to two levels of CD (540 vs. 360 cm<sup>2</sup>/hen) and three levels of PS (0, 0.15, and 0.30%). Probiotic contained *Enterococcus faecium* culture (10×10<sup>9</sup> cfu/g). Egg production (EP) and feed consumption (FC) were measured daily; egg weight (EW) was measured bi-weekly; BW was measured before and after the experiment, and blood samples were obtained at the end of the experiment. The data were analyzed using two-way ANOVA. Increasing CD decreased FC (125.0 vs. 120.8 g/d, p<0.0001) and FCR (1.93 vs. 1.87, p<0.0001) and did not alter EP, EW, and BW. Increasing level of PS linearly decreased FC (p<0.02) and FCR (p<0.006). Averages were 123.9, 123.2, and 121.6 g/d for FC and 1.91, 1.92, and 1.86 for FCR in hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. Hens placed in high-density cages had greater serum corticosterone concentration than hens placed in normal-density cages (12.8 vs. 11.3 µg/dL, p<0.04); CD did not affect concentrations of other metabolites. Increasing level of PS linearly increased serum glucose, albumin, and creatine concentrations and quadratically increased total protein, globulin, Ca, and P concentrations. Average concentrations (mg/dL) were 260, 297, and 305 for glucose; 6.28, 8.09, and 7.58 for total protein; 1.98, 2.48, and 2.38 for albumin; 4.30, 5.62, and 5.19 for globulin; 0.40, 0.52, and 0.54 for creatine; 16.0, 16.5, and 16.3 for Ca; and 6.27, 8.14, and 7.17 for P in hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. There was no effect of CD on egg quality. Increasing level of PS linearly improved yolk color (YC) and quadratically increased albumen index (AI) and Haugh unit (HU). The mean values were 9.67, 9.75, and 10.58 for YC; 8.94, 6.93, and 8.72% for AI; and 85.6, 74.9, and 82.9 for HU for hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. There was also CD by PS effect on FC, EP, and serum glucose, total protein, albumin, globulin, creatine, Ca and P concentrations. In conclusion, increased CD partially depressed laying performance and caused stress. Probiotic supplementation improved laying performance and metabolic profile. It also partially alleviated the adverse effects of stress resulting from increased caging density. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 12 : 1752-1760)

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

### INTRODUCTION

Despite seeming to be a practical approach to reduce investment cost in egg production, increasing cage density results in depressed laying performance (Hughes, 1975; Havenstein et al., 1989) and causes stress (Craig et al., 1986). Due to increased caging density, feeder space may be constrained, and consequently feed consumption and egg production are compromised (Cunningham and Gvaryahu, 1987; Brake and Peebles, 1992) as well as immune function may be impaired, and consequently health status is adversely affected (Cunningham, 1988; Cunningham et al., 1988; Odendaal, 1994). However, it is postulated that

depression in laying performance and poor welfare in response to increased caging density is more related to other housing conditions (i.e., temperature, humidity, and air quality) than increased caging density (Dawkins et al., 2004). Several nutritional practices have been employed to increase feed and/or nutrient intake and improve health status in hens housed at greater cage density. In response to increasing dietary protein concentration, alleviations in depressed laying performance in hens placed in high-density cages have been inconsistent (Bell et al., 1983; Moran, 1986). Supplemental lysine (Brake and Peebles, 1992) and subtherapeutic antibiotic (Dafwang et al., 1987) failed to improve laying performance and enhance health status of hens and chicks housed in high-density cages, respectively.

Probiotics is a generic term and refers to compounds containing yeasts and bacterial inoculants. Due to primarily their a lack of harmful effects on consumers (Onifade et al., 1999), inclusion of probiotics into poultry rations to enhance nutrient utilization, improve feed conversion efficiency, and maintain health status has become preferable

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**Table 1.** Ingredients of the experimental diets

Ingredient	Probiotic level (%)		
	0	0.15	0.30
Com	46.00	46.00	46.00
Soybean meal (44% CP)	21.00	21.00	21.00
Wheat	7.00	7.00	7.00
Barley	3.00	3.00	3.00
Wheat bran	8.75	8.60	8.45
Molasses	2.00	2.00	2.00
Limestone	9.00	9.00	9.00
Dicalcium phosphate <sup>1</sup>	2.00	2.00	2.00
Salt	0.40	0.40	0.40
Vitamin-mineral premix <sup>2</sup>	0.40	0.40	0.40
Methionine <sup>3</sup>	0.15	0.15	0.15
Lysine <sup>4</sup>	0.15	0.15	0.15
Ethoxyquin <sup>5</sup>	0.15	0.15	0.15
Probiotic <sup>6</sup>	-	0.15	0.30

<sup>1</sup> Each kilogram contained: Ca, 24% and P, 17.5%.

<sup>2</sup> Each kilogram contained: Vitamin A, 15,000 IU; cholecalciferol, 1,500 IU; DL- $\alpha$ -tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamine, 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 B<sub>12</sub>, 15  $\mu$ 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.

<sup>3</sup> DL-methionine. <sup>4</sup> L-lysine hydrochloride. <sup>5</sup> An antioxidant.

<sup>6</sup> Each kilogram ME-1-Roche contained 35 g cyclactin LBC SF-68ME with *Enterococcus faecium* culture at  $10 \times 10^9$  cfu/g.

during the last three decades (Dierck, 1989; Cavazzoni et al., 1998). Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora (Netherwood et al., 1999; Jadamus et al., 2001), enhancement of growth of non-pathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide (Ehrmann et al., 2002), suppression of growth of intestinal pathogens (Chateau et al., 1993; Jin et al., 1996; Fulton et al., 2002), enhancing immune status (Balevi et al., 2001), and improving nutrient availability and utilization (Nahashon et al., 1994; Schneitz et al., 1998; Jin et al., 2000). Probiotics were also shown to have binding property to mycotoxins (Byun and Yoon, 2003). These interconnected actions result in occurrence of acidic environment in intestine, increase in activity of intestinal enzymes, and detoxification of harmful metabolites, which lead to suppression of growth of pathogenic bacteria, enhancement of health status, and improvement in performance. Briefly, the major outcomes from using probiotics in broilers and growing chickens were shown to include improvements in growth (Yeo and Kim, 1997; Endo et al., 1999) and reduction in mortality rate (Kumprecht and Zobac, 1998) and feed conversion efficiency (Yeo and Kim, 1997). However, studies regarding effect of supplemental probiotic on performance of layers, especially those housed under stressing conditions, are limited. It was hypothesized that increasing cage density would depress laying performance and metabolic profile and deteriorate egg quality and probiotic supplementation would improve these parameters. Moreover, due to biopotent effects of probiotic

mentioned above, its supplementation would alleviate the adverse effects resulting from increased caging density. The aim of this experiment therefore was to evaluate the effects of cage density and probiotic 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 approved this experimental protocol. 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 or 540 vs. 360 cm<sup>2</sup>/hen) and three levels of probiotic (Cyclactin ME 1-ROCHE™, Roche Vitaminleri Ltd. Co., Istanbul, Turkey) supplementation (0, 0.15, or 0.30%) from wk 48 to 58. Each treatment was replicated in 6 cages. The experimental diets (Table 1) were formulated to be isocaloric and isonitrogenous and meet the NRC nutrient requirements (NRC, 1994). In the experimental groups, probiotic [Each kilogram ME-1-Roche contained 35 g cyclactin LBC SF-68ME with *Enterococcus faecium* culture at  $10 \times 10^9$  cfu/g] was added into the basal diet at expense of wheat bran (Table 1). The basal diet contained average of 89.0% DM and 2,690 Mcal/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. During the experimental period, hens were fed *ad libitum* once daily at 08:30 h and water through nipples was available all the times. Hens were also subjected to a 17L:7D cycle.

### 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 reported for chickens (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 (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced.

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 to determine metabolic profile. Serums were separated by centrifuging blood samples at 3,000 g for 15 min at 20°C.

**Table 2.** The effects of cage density and probiotic supplementation level on laying performance parameters during the during the peak production period in hens

Treatments		Response variables <sup>1</sup>							
Cage density (n) <sup>2</sup>	Probiotic 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	127.6	88.6	0.51	67.0	1.70	1.70	0.32	1.96
	0.30	121.3	89.8	0.71	64.4	1.58	1.62	-3.22	1.89
6 hens per cage	0	121.5	90.7	0.64	65.5	1.70	1.67	1.42	1.89
	0.15	118.8	86.8	0.30	65.4	1.66	1.65	0.20	1.88
	0.30	122.0	85.6	0.52	67.6	1.61	1.61	-0.16	1.83
SEM		1.0	1.2	0.21	2.1	0.04	0.03	1.52	0.02
ANOVA									
		----- p > F -----							
Cage density (CD)		0.0001	0.20	0.52	0.85	0.98	0.45	0.31	0.0001
Probiotic level (PL)		0.06	0.19	0.55	0.91	0.06	0.10	0.21	0.003
Linear effect		0.02	0.11	0.94	0.69	0.03	0.07	0.09	0.006
Quadratic effect		0.60	0.38	0.28	0.91	0.31	0.24	0.64	0.04
CD×PL		0.0001	0.02	0.75	0.33	0.63	0.72	0.57	0.51
Time (t)		0.0001	0.0001	0.31	0.19	-	-	-	0.0001
CD×T		0.0001	0.63	0.22	0.26	-	-	-	0.0001
PL×T		0.06	0.56	0.86	0.42	-	-	-	0.09
CD×PL×T		0.05	0.61	0.89	0.67	-	-	-	0.02

<sup>1</sup> Relative BW change = (initial BW-final BW)×100÷initial BW.

BW = body weight; FCR = feed conversion ratio (kg feed consumed per kg egg produced).

<sup>2</sup> 540 vs. 360 cm<sup>2</sup>/hen.

Aliquots were kept at -20°C until laboratory analyses for glucose, triglyceride, cholesterol, very low-density lipoprotein, total protein, albumin, globulin creatine, Ca, and P concentrations using commercial kits (DDS<sup>®</sup>, Dias Diagnostic Systems Co., Istanbul 80270, Turkey) as well as corticosterone concentration using RIA (Beuving and Vonder, 1977). Intraassay CV for hormone assay was 12.8%.

To assess egg quality parameters, another sample of three eggs was randomly collected from 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/cm<sup>2</sup>) was determined by using machine with the spiral pressure system, shell thickness (mm×10<sup>-2</sup>) 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 colour was determined by using commercially available yolk colour 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<sup>0.37</sup>), where AH = albumen height, mm and EW = egg weight, g.

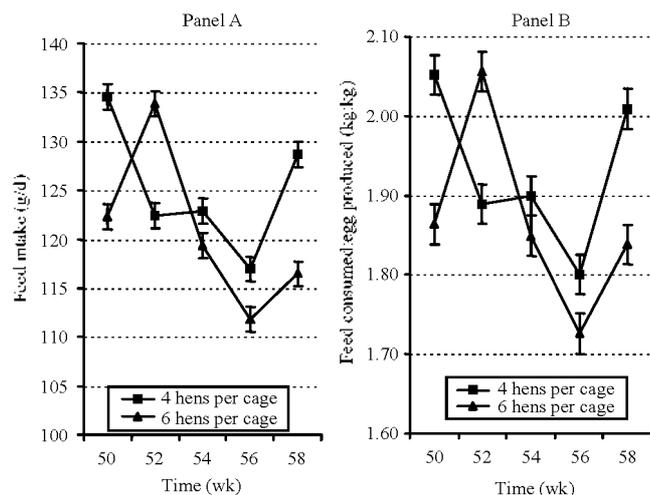
### Statistics

Treatments were set by factorial arrangements of two

caging densities and three probiotic supplementation levels and 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:

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

where,  $Y_{ijkl}$  = response variable,  $\mu$  = population mean,  $b_0$  = intercept,  $b_1$  = slope,  $\text{Cov}_{\text{BW}}$  = covariate (BW = body weight),  $B_i$  = block ( $i = 1^{\text{st}}$  cage at lower level by aisle to  $6^{\text{th}}$  cage at upper level by window),  $CD_j$  = cage density ( $j = 4$  or 6 hens per cage),  $PL_k$  = probiotic level ( $k = 0$  to 0.3%),  $(CD \times PL)_{jk}$  = cage density  $j$  and probiotic 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,  $(PL \times T)_{kl}$  = probiotic level  $k$  and time  $l$  interaction,  $(CD \times PL \times T)_{jkl}$  = cage density  $j$  and probiotic level  $k$ , and time  $l$  interaction, and Error B = subplot error.



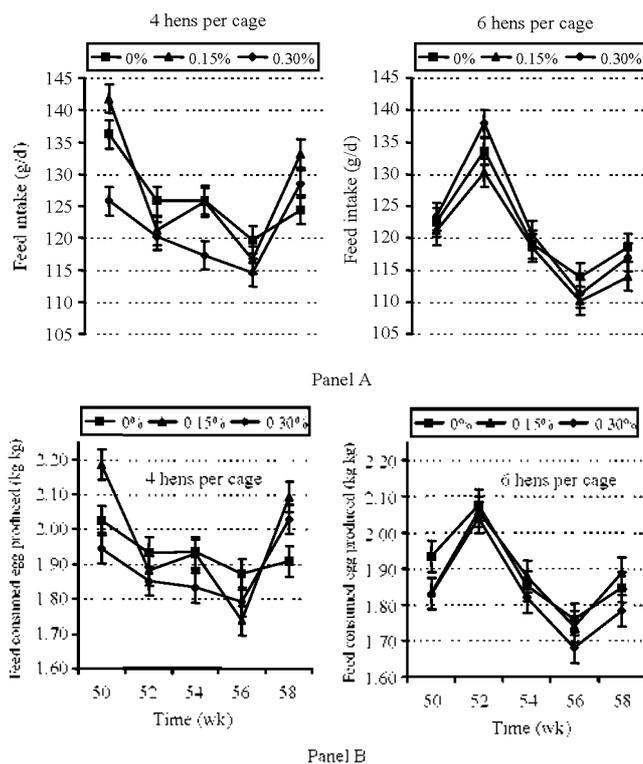
**Figure 1.** The effects of cage density by time interaction on feed consumption (SEM = 1.28 and  $p < 0.0001$ ) [Panel A] and FCR (SEM = 0.03 and  $p < 0.0001$ ) [Panel B].

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

## RESULTS

### Laying performance

Table 2 shows the effects of cage density and probiotic supplementation level on laying performance. The cage density affected feed consumption and feed conversion ( $p < 0.0001$  for both), but did not alter other laying performance parameters. Hens placed in normal-density cages had greater daily feed consumption (125.0 vs. 120.8 g/d) and consumed greater amount of feed per kilogram of egg production (1.93 vs. 1.87 kg) than hens placed in high-density cages. Feed consumption ( $p < 0.02$ ) and feed conversion ( $p < 0.006$ ) decreased linearly, whereas other laying performance parameters did not change with increasing level of probiotic supplementation. The mean values were 123.9, 123.2, and 121.6 g/d for feed consumption and 1.91, 1.92, and 1.86 for FCR in hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. There were also cage density by probiotic level interaction effects on feed consumption ( $p < 0.0001$ ) and hen-day egg production ( $p < 0.02$ ). Feed consumption for hens placed in normal-density cages decreased, whereas that for hens placed in high-density cages increased as supplemental probiotic level increased. Hen-day egg production for hens placed in normal-density cages increased, whereas that for hens placed in high-density cages decreased as supplemental probiotic level increased (Table 2).



**Figure 2.** The effects of three-way interaction of cage density, probiotic supplementation level, and time on feed consumption (SEM = 2.21 and  $p < 0.05$ ) [Panel A] and FCR (SEM = 0.04 and  $p < 0.02$ ) [Panel B].

Feed consumption, hen-day egg production, and feed conversion varied ( $p < 0.0001$ ) as the experiment continued. The mean values were 128.5, 128.1, 121.1, 114.3, and 122.6 g/d for feed consumption; 82.8, 89.1, 89.3, 89.6, and 90.7% for hen-day egg production; and 1.96, 1.97, 1.87, 1.76, and 1.92 for FCR on wk 50, 52, 54, 56, and 58, respectively. As the experiment continued, cage density, but not probiotic supplementation affected feed consumption and feed conversion differently ( $p < 0.0001$  for both; Figure 1, Panels A and B). Both parameters for hens placed in normal-density cages continuously decreased and then increased towards the end of the experiment, whereas those for hens placed in high-density cages first increased, later continuously decreased, and then increased towards the end of the experiment. Moreover, there was also three-way interaction effect of caging density, probiotic supplementation level and time on feed consumption ( $p < 0.05$ ; Figure 2, Panel A) and feed conversion ( $p < 0.02$ ; Figure 2, Panel B). Increasing level of probiotic supplementation, quadratically decreased feed consumption and feed conversion for hens placed in normal-density cages, whereas linearly decreased these parameters for hens placed in high-density cages as the experiment continued.

### Metabolic profile

Table 3 summarizes the effects of caging density and

**Table 3.** The effects of cage density and probiotic supplementation level on metabolic profile during the during the peak production period in hens

Treatments		Response variables <sup>1</sup>										
Cage density (n) <sup>2</sup>	Probiotic level (%)	Cort	Glc	TG	Chol	VLDL	TP	Alb	Glo	Cre	Ca	P
4 hens per cage	0	11.06	239.3	827.7	159.5	165.5	5.60	1.73	3.87	0.38	15.65	5.43
	0.15	11.08	281.8	820.2	164.5	164.2	6.98	2.28	4.70	0.47	16.30	7.25
	0.30	12.04	334.8	824.8	186.0	164.8	8.42	2.57	5.85	0.57	16.47	8.82
6 hens per cage	0	12.46	280.5	819.3	203.8	163.8	6.95	2.22	4.73	0.42	16.32	7.10
	0.15	13.16	312.5	815.8	237.3	163.3	9.20	2.67	6.53	0.58	16.67	9.03
	0.30	12.88	274.7	815.3	173.0	163.0	6.73	2.20	4.53	0.52	16.17	5.52
SEM		0.84	13.2	6.9	27.2	1.4	0.47	0.11	0.37	0.03	0.19	0.79
ANOVA												
		p > F										
Cage density (CD)		0.04	0.72	0.20	0.13	0.20	0.11	0.08	0.14	0.23	0.12	0.94
Probiotic level (PL)		0.71	0.004	0.72	0.69	0.77	0.002	0.0001	0.004	0.0001	0.04	0.08
Linear effect		0.41	0.002	0.62	0.94	0.58	0.009	0.001	0.02	0.0001	0.09	0.26
Quadratic effect		0.98	0.20	0.53	0.40	0.65	0.007	0.006	0.01	0.07	0.05	0.05
CD×PL		0.79	0.001	0.93	0.29	0.92	0.001	0.001	0.001	0.05	0.05	0.004

<sup>1</sup> 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 ( $\mu\text{m}/\text{dl}$ ).

<sup>2</sup> 540 vs. 360 cm<sup>2</sup>/hen.

**Table 4.** The effects of cage density and probiotic supplementation level on egg quality parameters during the during the peak production period in hens

Treatments		Response variables						
Cage density (n) <sup>2</sup>	Probiotic level (%)	Shape index (%)	Shell strength (kg/cm <sup>2</sup> )	Shell thickness (mm×10 <sup>-2</sup> )	Yolk color	Yolk index (%)	Albumen index (%)	Haugh unit
4 hens per cage	0	72.6	1.008	1.090	9.33	39.2	8.88	84.3
	0.15	74.8	1.373	1.072	10.33	39.0	6.85	76.0
	0.30	74.3	1.132	1.088	10.67	38.1	9.23	84.5
6 hens per cage	0	74.2	1.122	1.048	10.00	41.4	9.00	86.8
	0.15	73.4	1.537	1.107	9.17	38.1	7.01	73.7
	0.30	72.9	1.335	1.065	10.50	40.4	8.20	81.3
SEM		0.9	0.208	0.028	0.45	0.9	0.51	2.5
ANOVA								
		p > F						
Cage density (CD)		0.59	0.36	0.66	0.55	0.11	0.55	0.63
Probiotic level (PL)		0.71	0.19	0.76	0.10	0.17	0.001	0.001
Linear effect		0.78	0.43	0.79	0.05	0.25	0.67	0.30
Quadratic effect		0.44	0.10	0.50	0.34	0.13	0.0001	0.0001
CD×PL		0.16	0.98	0.36	0.14	0.15	0.42	0.48

<sup>1</sup> 540 vs. 360 cm<sup>2</sup>/hen.

probiotic supplementation level on metabolic profile. Except for serum corticosterone concentration, other metabolic parameters were not affected by cage density. Hens placed in high-density cages had greater serum corticosterone concentration (12.8 vs. 11.3  $\mu\text{g}/\text{dl}$ ,  $p < 0.04$ ) than hens placed in normal-density cages. Increasing level of probiotic supplementation linearly increased serum glucose ( $p < 0.002$ ), albumin ( $p < 0.001$ ), and creatine ( $p < 0.008$ ) concentrations and quadratically increased total protein ( $p < 0.007$ ), globulin ( $p < 0.002$ ), Ca ( $p < 0.05$ ), and P ( $p < 0.05$ ) concentrations. The mean serum concentrations (mg/dl) were 260, 297, and 305 for glucose; 6.28, 8.09, and 7.58 for total protein; 1.98, 2.48, and 2.38 for albumin; 4.30, 5.62, and 5.19 for globulin; 0.40, 0.52, and 0.54 for

creatinine; 16.0, 16.5, and 16.3 for Ca; and 6.27, 8.14, and 7.17 for P concentrations in hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. Moreover, there was a cage density by probiotic level interaction effect on serum glucose, total protein, albumin, globulin, creatine, Ca, and P concentrations. With increasing level of supplemental probiotic, all these parameters increased linearly for hens placed in normal-density cages, whereas they increased quadratically for hens placed in high-density cages.

#### Egg quality

Egg quality parameters in response to different densities of cage and levels of supplemental probiotic are shown in Table 4. Increased caging density did not affect egg quality

parameters (Table 4). As supplemental probiotic level increased, yolk color improved linearly ( $p < 0.05$ ) and albumen index and Haugh unit increased quadratically ( $p < 0.0001$  for both). The mean values were 9.67, 9.75, and 10.58 for yolk color; 8.94, 6.93, and 8.72% for albumen index; and 85.6, 74.9, and 82.9 for Haugh unit for hens supplemented with 0, 0.15, and 0.30% probiotic, respectively. There was no cage density by probiotic level interaction effect on egg quality parameters.

## DISCUSSION

Numerous studies have performed to evaluate the effects of a great range of stocking densities on laying performance and conflicting results from these studies appear to depend on the degree of density (Hughes, 1975), and consequently of limitation of physical activity (Brake and Peebles, 1992) and disturbance of social behaviors (Cunningham, 1988). In the present study, no death or aggressive behavior was observed because of increasing caging density. Also, feed consumption depressed and FCR improved and no changes in egg production, BW, and egg weight occurred in response to increased caged density (Table 2). At the densities of 450, 525, 600 and 750 cm<sup>2</sup> area per hen, Bishop (2004) reported no changes in laying performance parameters. Depression in feed consumption in this experiment could be attributed to restricted feeder space or increased heat production. In agreement with the present data, other researchers (Ramos et al., 1986; Cunningham and Gvoryahu, 1987; Brake and Peebles, 1992) also reported a depression in feed consumption for hens housed at increased caging densities. Because of a lack of cage density effect on hen-day egg production and egg weight in the present experiment, increased caging density was associated with improvements in FCR, which contradicts with results of previous studies (Roush et al., 1984; Brake and Peebles, 1992; Lee and Moss, 1995a). However, other studies involving hens housed at high-cage density reported reductions in hen-day egg production (Roush et al., 1984; Ramos et al., 1986; Lee and Moss, 1995a; Sohail et al., 2001), BW (Ramos et al., 1986; Cunningham and Gvoryahu, 1987), and egg weight (Sohail et al., 2001), and mortality (Craig and Milliken, 1989; Moinard et al., 1998), and increase in cracked egg yield (Hester and Wilson, 1986). In these studies, caging density ranged from 1,394 to 310 cm<sup>2</sup> area per hen. General industry standard of caging density is 450 cm<sup>2</sup> per hen and the adverse effects appear to become significant in area per hen less than 697 cm<sup>2</sup> (Mench et al., 1986).

As mentioned earlier, mechanism by which probiotic affects poultry performance is well established. Also, due to public concern against antibiotic usage, there is a growing interest in utilization of biotechnological products for

animal health and production efficiency (Onifade et al., 1999). Studies involving chickens (Harms and Miles, 1988; Grimes et al., 1997; Fairchild et al., 2001), turkeys (Waldroup et al., 1972), and quails (Davis, 1996; Davis and Qureshi, 1997) showed that there were improvements in BW, egg production, and immune function in response to supplemental bacterial cultures. It appears that supplementations of probiotic do not improve growth by affecting feed intake per se, suggesting that improvement in weight gain and reduction in feed conversion efficiency by supplemental probiotic could be related to its promoting effects on metabolic processes of digestion and utilization of nutrients and enhancing health status (Yeo and Kim, 1997; Jin et al., 1998). In agreement with the present experiment (Table 2), Mohan et al. (1995) reported a quadratic increase in egg production in chickens supplemented with 0, 100, and 150 mg probiotic (*Lactobacillus*, *Bifidobacterium*, *Aspergillus*, *Torulopsis* spp. at  $27 \times 10^9$  cfu/10 g) per kilogram diet during the peak period. Yörük et al. (2004) supplemented late laying hens (54 wks of age) for three months with 0.1 and 0.2% probiotic and reported a linear increase in egg production and linear decreases in mortality rate, feed intake, and feed conversion ratio. However, growth-promoting effects of supplemental probiotic have not been evaluated in laying hens exposed to stressing conditions intensively. Davis and Anderson (2002) housed hens at 310 and 413 cm<sup>2</sup> per hen and fed laying hens probiotic containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium thermophilum* ( $1.0 \times 10^8$  cfu/g) at dose of 454 g per ton of the diet. Probiotic provision increased egg weight and percentage of large egg. It also alleviated depression in egg weight, not hen-housed egg production resulting from increased caging density, suggesting that probiotic can improve egg size and lower feed costs, regardless of the bird density. Lan et al. (2004) exposed broilers to acute heat stress and supplemented with *Lactobacillus agilis* and *salivarius* strains. The probiotic supplementation enriched the diversity of *Lactobacillus* spp. in jejunum and cecum by increasing the abundance and prevalence of *Lactobacillus* spp. inhabiting the intestine, restored the microbial balance, and maintained the natural stability of indigenous bacterial flora. In a comparison of probiotic (*Lactobacillus* culture, 50 mg/kg) and antibiotic (oxytetracycline, 1 g/kg) supplementations in broilers reared under high ambient temperature, it was shown that these supplements improved BW gain and FCR until day 21 when heat introduced. During the exposure to heat, birds fed probiotic had greater BW and feed consumption and lower FCR than those fed antibiotic and the control diet. However, dietary treatments did not differ antibody production against Newcastle disease (Zulkifli et al., 2000).

The effect of increased caging density on stress is

controversial and depends on the degree of stocking density (Okpokho et al., 1987; Cunningham, 1988; Lee and Moss, 1995b). Corticosterone is considered as an indicator of stress (Post et al., 2003) and the major steroid hormone released by the avian adrenal gland (Culbert and Wells, 1975). In this study, only corticosterone concentration was affected by increased caging density, and other blood metabolites did not refer any indication of catabolic activities resulting from crowding (Table 3). It is shown that exposure to stress increases serum glucose (Saleh and Jaksch, 1977) and corticoid (Mashaly et al., 1984; Mench et al., 1986; Davis et al., 2000) concentrations and impairs immune function (Heckert et al., 2002). Some studies however reported no change in serum corticosterone concentrations of hens housed at increasing caging densities (Koelkebeck and Cain, 1984; Bishop, 2004). The ratio of heterophils to lymphocytes is relatively more stable and considered to be sensitive criterions of stress (Gross and Siegel, 1983), but not measured in the present study. In disagreement with the present results, other studies reported elevation in serum triglyceride (Jensen et al., 1976) and Ca (Moinard et al., 1998) and decrease in serum cholesterol (Bishop, 2004) concentrations in hens housed at increased caging densities.

Probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients (Dierck, 1989; Schneitz et al., 1998), which may result in increased feed consumption and be reflected as changes in metabolic profile (Table 3). In disagreement with the present experiment, Jin et al. (1998) reported that increasing level of supplemental *Lactobacillus* culture up to 0.10% with 0.05% increments linearly decreased serum cholesterol level in broilers. Endo et al. (1999) also reported that supplemental probiotic (a mixture of *Bacillus*, *Lactobacillus*, *Streptococcus*, *Clostridium*, *Saccharomyces* and *Candida*) altered the lipid metabolism and consequently reduced serum cholesterol concentration. Moreover, cage density by probiotic interaction effect on carbohydrate, protein and mineral metabolism parameters may suggest alteration of nutrient partitioning and alleviation of stress (Table 3). Probiotics exert antioxidative properties by reducing glutathione peroxidase activity and inhibiting linoleic acid peroxidation, suggesting that probiotic supplementation may alleviate the adverse effect of stress on welfare (Yoon and Byun, 2004).

Similar to the present experiment (Table 4), 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). However, Altan et al. (2002) and Bishop (2004) reported that except for a linear increase in Haugh unit, other egg quality parameters did not change in hens housed at increased caging densities. Unlike the present study, other

researchers (Hester and Wilson, 1986; Mench et al., 1986) reported that increasing cage density caused shell strength. Data on the effect of supplemental probiotic on egg quality are limited. Alike our previous experiment (Yörük et al., 2004), only albumen index and Haugh unit were affected by probiotic supplementation. Williams (1992) reviewed factors that affect albumen height and concluded that a few nutritional factors have an effect, but, overall, nutrition is relatively unimportant. Some genetic (i.e., strain and age) and management (i.e., egg storage time and storage conditions) factors influence these interrelated parameters ( $r = 0.92$ ,  $p < 0.0001$ ) that are indicator of grading and freshness of eggs. Improvement in yolk color in response to increasing level of probiotic supplementation in this present experiment is unexplainable. In disagreement with the present experiment, Mohan et al. (1995) reported a slight improvement in eggshell thickness in response to 10-week probiotic supplementation.

## CONCLUSIONS

The effects of cage density and probiotic supplementation on laying performance, metabolic profile, and egg quality parameters were evaluated in peak producing hens. Increasing caging density decreased feed consumption, improved FCR, and did not alter hen-day egg production, BW, and egg weight as well as egg quality parameters. Increasing probiotic supplementation up to 0.30% improved these laying performance parameters. Increase in corticosterone concentration revealed that increasing caging density caused physiological stress. Probiotic supplementation alleviated the adverse effects of increased caging density on feed consumption and hen-day egg production. It also affected serum carbohydrate, protein, and mineral metabolites. Increasing level of supplemental probiotic improved yolk color, albumen index and Haugh unit. Briefly, increased caging density was associated with stress and depression in feed intake. Supplemental probiotic improved laying performance and metabolic profile. Moreover, supplemental probiotic partially alleviated depressions in laying performance and metabolic profile resulting from increased caging density.

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