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Effects of Breeder Age and Stocking Density on Performance, Carcass Characteristics and Some Stress Parameters of Broilers

E. E. Onbaşılar*, Ö. Poyraz and S. Çetin[1]

Ankara University, Faculty of Veterinary Medicine, Department of Animal Science, 06110, Ankara, Turkey

ABSTRACT : The aim of this study was to determine the effects of breeder age and stocking density on performance, carcass characteristics and some stress parameters (H-L ratio, serum glucose, cholesterol and triglyceride levels, tonic immobility test (TI), antibody production, relative asymmetry (RA) and external appearances). This experiment was carried out with 705 one-day old male broiler chicks (Ross 308) obtained from three different ages of broiler breeder (32, 48 and 61 wks). Each age group was randomly divided into two stocking density groups (11.9 and 17.5 broilers per m^2) with 5 replications per group. The experimental period was 6 weeks. Broilers from 32 wk-old breeders had lower initial weight ($p<0.001$), body weight gain of the first 3 week of rearing ($p<0.01$), the percentage of abdominal fat ($p<0.001$) and serum cholesterol level ($p<0.01$); higher percentage of gizzard ($p<0.01$) and longer TI duration ($p<0.001$) than those from 48 and 61 wk-old breeders. Broilers reared at 17.5 b/m^2 had lower final BW, body weight gain, feed consumption, feather condition and foot health ($p<0.001$), higher percentage of heart, H-L ratio, serum glucose and cholesterol levels ($p<0.001$), and longer TI durations ($p<0.001$). There were no significant interactions in examined parameters except for feed to gain ratio between breeder age and stocking density. (**Key Words :** Breeder Age, Stocking Density, Broiler, Stress)

INTRODUCTION

Young breeder flocks are often reported to produce eggs with low hatching potential, extended incubation periods, and chicks of low quality as judged by subsequent mortality and growth. For example, mortality was significantly higher among chicks coming from a 26 wk old flock compared with chicks from a 36 wk old broiler breeder flock, according to Wyatt et al. (1985). The most obvious characteristics of eggs from young broiler breeders are low egg weight. Because chick BW is proportional to egg weight, small chicks are to be expected from young breeder.

Older hens lay larger eggs that hatch into larger chicks (Wilson, 1991), and egg weight and hatching weight of chicks are correlated with market age weight. Small chicks from young hens have higher mortality after placement and reach market weight at a later age. A one-g increase in hatching weight has been found to result in increased weight at market age (Shanawany, 1987).

* Corresponding Author: E. E. Onbaşılar. Tel: +90-312-3170315-311, Fax: +90-312-3181758, E-mail: obasilar@veterinary.ankara.edu.tr

[1] Atatürk University, Faculty of Veterinary Medicine, Department of Microbiology, 25240, Erzurum, Turkey.

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To achieve their genetic potential for growth, broilers must be provided with optimal environmental conditions. Any deviation from optimal conditions can result in decreased performance. High stocking densities can contribute to reduced performance due to a number of factors. The overall effect on broiler chickens of reducing floor space can be reduced growth rate, feed efficiency, livability, and, in some cases, carcass quality (Puron et al., 1995).

Stress has been assessed from performance, relative asymmetry (RA), tonic immobility (TI), some blood parameters (heterophil-lymphocytes ratio (H/L), glucose, cholesterol and triglyceride levels) and immune response. Because H-L ratio was increased under stressful conditions, the ratio has been used as an index of the responses of hypothalamic-hypophyseal-adrenal axis to the stressors (Gross and Siegel, 1983). Genetic and environmental stressors may increase asymmetry of bilateral traits in poultry (Yalçın et al., 2003). Tonic immobility (TI) is an adaptive psycho-physiological response characterized by reduced responsiveness induced by physical restraint and has been widely used a measure of fearfulness in poultry (Jones and Faure, 1980).

However, information in the literature regarding the effects of breeder age and stocking density on performance,

Table 1. Composition of the diets (g/kg)

	Starter diet (0-21 d)	Grower diet (22-42 d)
Corn	440.0	538.0
Soyabean meal	268.0	200.0
Full-fat soya	180.0	160.0
Meat and bone meal	40.0	40.0
Soyabean oil	38.5	28.5
Limestone	15.0	15.0
Dicalcium phosphate	12.0	12.0
Salt	2.5	2.5
Vitamin-mineral premix[a]	2.5	2.5
DL-methionine	1.5	1.5
Chemical composition (Analyzed)		
Crude protein (g/kg)	229	201
ME (MJ/kg)	13.25	13.21

[a] Composition per 2.5 kg: 12,000,000 IU vitamin A, 3,000,000 IU vitamin D_3, 30 g vitamin E, 3.75 g vitamin K_3, 3 g vitamin B_1, 6 g vitamin B_2, 5 g vitamin B_6, 20 mg vitamin B_{12}, 40 g niacin,10 g cal-D-panthotenate,1 g folic acid, 50 mg D-biotin, 500 g choline chloride, 80 g Mn, 60 g Fe, 60 g Zn, 5 g Cu, 0.5 g Co, 1 g I, 0.15 g Se.

carcass characteristics and some stress parameters are limited. This study was, therefore, conducted to determine the effects of breeder age and stocking density on performance, carcass characteristics and some stress parameters (H-L ratio, serum glucose, cholesterol and triglyceride levels, tonic immobility test (TI), antibody production, relative asymmetry (RA) and external appearances).

MATERIALS AND METHODS

Hatching eggs, animals and diets

Eggs were obtained from three different ages (32, 48 and 61 wks) of broiler breeders (Ross 308). These flocks were housed under the same environmental and management conditions (20°C, 55% RH and 16L:8D) at the different closed houses in the same farm. All birds received the same mash broiler breeder-laying diet (11.51 MJ/kg ME, 160 g/kg crude protein, 3.7% calcium and 0.45% available phosphorus). Eggs were collected at the same time (between 1000 and 1100 h) from different houses and were stored for one day at 20°C and 75% RH. After the storage period, all eggs were incubated (Model 576, Petersime) at 37.3°C dry bulb temperature and 30°C wet bulb temperature, then at 18 days, eggs were transferred to the hatcher (Model 192, Petersime). The hatcher was operated at 36.8°C dry bulb temperature and 30°C wet bulb temperature. Chicks were removed at 21 d of incubation. 235 male chicks from each age of broiler breeder flocks (a total of 705) were selected at random. These chicks from each age of breeder groups were randomly divided into 2 stocking density groups (11.9 and 17.5 broilers per m^2). The number of chicks was 19 and 28 in stocking groups of 11.9 b/m^2 and 17.5 b/m^2, respectively. There were 5 replicate pens in breeder age and stocking density group. Chicks of each group were placed in separate floor pen measured as 170×94×90 cm, width×length×height, respectively. Each pen had wood shavings litter, two nipples and one hanging suspended feeder. Experiment was conducted during the spring (April to May) season in windowed house. Birds were fed (mash form) with a starter diet from 1 to 21 d of age (13.25 MJ/kg ME, 229 g/kg crude protein) and grower diet from 22 to 42 d of age (13.21 MJ/kg ME, 201 g/kg crude protein). Compositions of diets in starter and grower periods of the experiment were presented in Table 1. Feed and water were available *ad libitum* during the experiment. Birds were provided with continuous light. Average pen temperatures were 32°C on the first week then gradually lowered to average 24°C and this temperature was maintained up to slaughter age. This average temperature was supplied with stove of hazelnut. During the experiment, house temperature was measured four times a day. Birds were vaccinated against Newcastle disease at hatching and again at 4 weeks; against infectious bronchitis at hatching and again at 10 days of age; and against infectious bursal disease at 18 and 24 days of age.

Traits measured

Every week all broilers were weighed. Feed consumption and feed to gain ratio were measured weekly too. At 3 days of age 10 chicks from each pen were tested individually on the T-maze apparatus. This apparatus (described by Marin et al., 1997) consists of an isolation chamber measuring 21×21 cm (length×width) leading to a 21 cm long×7 cm wide T corridor with 7×7 cm perpendicular arms. A 10×10 cm mirror was situated at the junction of the T corridor to facilitate movement of the chick towards this point. Light was provided by a 100-watt overhead lamp. At test, each chick was placed individually in the centre of the isolation box facing away from the entrance to the T corridor. Its escape time was recorded. Then chick was immediately returned to its pen.

At day 31, ten broilers were randomly selected from each pen and injected with 0.1 ml of 0.25% suspension of sheep erythrocytes (SRBC) in phosphate buffer saline (PBS). Circulating anti-SRBC antibody titers were determined by the microhemagglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution (Ruiz-Feria and Abdukalykova, 2006). Ten broilers were randomly selected from each pen at day 36 and bled from the brachial vein, then anti NDV titer was determined. Serum anti-NDV titer was assayed using the hemagglutination inhibition method (Allan and Gough, 1974).

At d 40, for each pen, ten broilers randomly selected were assessed for TI, RA and external appearance. TI was measured as described by Jones and Faure (1980). Broiler was carried to a separate and quiet room, to avoid unnecessary cumulative stress, which might be associated with catching, handling and moving the birds. Each chicken was carefully restrained for 15 s by covering the head with one hand, while placing the other hand on the sternum. Latency to self-righting was used as the measure of TI. If this had not happened after 10 min, this session was terminated and the individual was assigned a value of 600 s. If the chicken terminated the state of immobility before 10 s, the trial was repeated. Observations were performed at a distance of 2-2.5 m without making unnecessary noise and movements. After the TI test, RA was measured as the length of the right and left tarsometatarsus and metatarsus and the width of the tarsometatarsus and metatarsus at the spur were recorded to the nearest 0.1 mm using digital calibers. Trait size was the mean of the left and right sides. RA was defined as the ratio of the absolute value of the left minus right divided by the value for the size of the trait. Mean RA was defined as the mean RA of the different traits. After these measures, external appearance traits including feather condition, skin injuries, claw length and foot health of five birds per pen were measured. Birds were scored for the feather condition and skin injures of five individual parts of the body, i.e., neck, breast, back, wings and tail. A score (graduated from 1 = very poor feather to 4 = intact plumage) was assigned for feather condition for each area of the body parts. Feather conditions of all parts were added and calculated as feather condition of broiler (5-20). Skin injuries were scored from 1 to 4 points, where 1 = large defects on skin and 4 = no defects. Claw length was measured with digital caliper. Foot health was scored on a 1 to 4 scale, where 1 = severely injured matrix and 4 = intact matrix (Tauson, 1984).

At d 41, ten broilers from each pen were randomly selected and bled from the brachial vein. Blood samples were taken in two tubes, one contained EDTA for estimating the H-L ratio, and the other had no anticoagulant for estimating cholesterol, glucose and triglyceride levels. The bleeding procedure was limited to 1 min or less to minimize the influence of handling stress. Blood samples were smeared on to a glass slide for the determination of the H-L ratio. After drying, the smears were stained with May-Grünwald-Giemsa stain (Gross and Siegel, 1983). One hundred leucocytes were counted on each slide, using a light microscope at ×1,000 magnification. The H-L ratios were determined by dividing the number of heterophils by the number of lymphocytes. Serum cholesterol, glucose and triglyceride levels were determined using a Vitros 350 auto-analyzer (New York, USA; Product code 680-2153) and its accompanying commercial kits.

At 42 d of age, five birds of each pen (25 birds of each age and density group) were randomly selected for processing. After final weighing feed was removed 6 h prior to slaughter. Slaughtering is conducted by cutting the jugular veins and carotid arteries. Carcass, abdominal fat, bursa of Fabricius, heart, liver and spleen were weighed. These weights were expressed as percentage of slaughter weight. The carcasses were stored at 4°C for 20 h by hanging. Cold carcass weights were recorded and were expressed as percentage of slaughter weight as cold carcass yield. Each carcass was cut into its three component parts containing muscle with skin and bone: wings, saddle with legs and breast with rack (Havenstein et al., 2003). They were weighed and expressed as a percentage of cold carcass weight.

Statistical analyses

Statistical analyses were performed by using software package SPSS for Windows (SPSS Inc, Chicago, IL, USA). Data were tested for distribution normality and homogeneity of variance. A two-way ANOVA was used to determine the differences between breeder age and stocking density and their interactions with respect to the studied parameters. When a significant difference was found among groups for post-hoc multiple comparisons, Duncan test was used (Dawson and Trapp, 2001).

RESULTS AND DISCUSSION

Average chick hatching weight was lower ($p<0.001$) from younger breeders (Table 2). Similarly, Noble et al. (1986) reported that smaller eggs produced by very young (25 wk of age) broiler breeder hens have been found to yield smaller chicks with longer residual yolk sacks than breeders at 41 wk.

Body weight gain of the first 3 week of rearing was lower for broilers from younger breeders ($p<0.01$). But from the 4 to 6 wks of age, body weight gain and final body weight were similar among breeder age groups. Contrary Peebles et al. (1999a) showed that there was a significant breeder age effect for broiler BW gain between 0-21, 21-42 and 0-42 d of broiler age. They reported that broilers from hens at 35 wk of age were lowest BW gain than in those at 51 and 63 wk of age. Feed consumption and feed to gain ratio were not affected from breeder age in this study.

Body weight gain, feed consumption and final body weight were decreased with increasing stocking density from 11.9 b/m^2 to 17.5 b/m^2. This reduction in growth rate was due to the decrease in feed consumption. Feed to gain ratio at wk from 1 to 6 was not statistically significant between stocking density groups. Similarly, Feddes et al. (2002) reported that as stocking density increased from 14 to 18 b/m^2 cumulative BW and feed consumption were decreased with broilers weighed approximating 1.9 kg. Shanawany (1988) reported that when using 1.8 kg broilers

Table 2. Effects of breeder age and stocking density on initial weight, final weight, body weight gain, feed consumption and feed to gain ratio of broilers

BA (wk)	SD (b/m^2)	Initial weight (g)	Final weight (g)	Body weight gain (g)			Feed consumption (g)			Feed to gain ratio (g/g)		
				Wks from 1 to 3	Wks from 4 to 6	Wks from 1 to 6	Wks from 1 to 3	Wks from 4 to 6	Wks from 1 to 6	Wks from 1 to 3	Wks from 4 to 6	Wks from 1 to 6
32												
	11.9	40.8±0.3	2,494±31	694±11	1,760±24	2,453±31	865±23	2,864±50	3,729±54	1.25±0.03	1.63±0.02	1.52±0.02
	17.5	40.1±0.3	2,244±31	678±11	1,526±24	2,204±31	834±23	2,671±50	3,506±54	1.23±0.03	1.75±0.02	1.59±0.02
48												
	11.9	46.0±0.3	2,545±31	742±11	1,756±24	2,499±31	943±23	2,942±50	3,884±54	1.27±0.03	1.68±0.02	1.56±0.02
	17.5	46.6±0.3	2,288±31	716±11	1,525±24	2,241±31	815±23	2,690±50	3,505±54	1.14±0.03	1.76±0.02	1.56±0.02
61												
	11.9	47.7±0.3	2,500±31	722±11	1,730±24	2,453±31	910±23	2,960±50	3,870±54	1.26±0.03	1.71±0.02	1.58±0.02
	17.5	47.1±0.3	2,306±31	710±11	1,549±24	2,259±31	854±23	2,668±50	3,522±54	1.20±0.03	1.72±0.02	1.56±0.02
Total												
	11.9	44.8±0.2	2,513±18^x	720±6^x	1,748±14^x	2,468±18^x	906±13^x	2,922±29^x	3,828±31^x	1.26±0.02^y	1.67±0.01^y	1.55±0.01
	17.5	44.6±0.2	2,279±18^y	701±6^y	1,533±14^y	2,235±18^y	834±13^y	2,676±29^y	3,511±31^y	1.19±0.02^x	1.75±0.01^x	1.57±0.01
32		40.4±0.2^c	2,369±22	686±8^b	1,643±17	2,329±22	850±16	2,768±35	3,618±38	1.24±0.02	1.69±0.02	1.56±0.01
48		46.3±0.2^b	2,416±22	729±8^a	1,641±17	2,370±22	879±16	2,816±35	3,694±38	1.20±0.02	1.72±0.02	1.56±0.01
61		47.4±0.2^a	2,403±22	716±8^a	1,640±17	2,356±22	882±16	2,814±35	3,696±38	1.23±0.02	1.72±0.02	1.57±0.01
Two way ANOVA (p)												
SD		0.437	0.000	0.053	0.000	0.000	0.001	0.000	0.000	0.014	0.000	0.179
BA		0.000	0.310	0.002	0.993	0.411	0.322	0.563	0.265	0.489	0.343	0.748
SD×BA		0.103	0.550	0.810	0.481	0.541	0.114	0.614	0.324	0.220	0.050	0.050

Values are mean±SEM. $^{a, b, c, x, y}$ Means within columns with different letters are significantly different ($p<0.05$).

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction.

Table 3. Effects of breeder age and stocking density on carcass characteristics of broilers

BA (wk)	SD (b/m^2)	Hot carcass yield (% BW)	Cold carcass yield (% BW)	Legs and saddle (% CC)	Breast and rack (% CC)	Wings (% CC)
32						
	11.9	73.47±0.47	72.35±0.48	41.60±0.30	41.49±0.36	11.25±0.14
	17.5	72.90±0.47	71.70±0.48	42.11±0.30	40.70±0.36	11.27±0.14
48						
	11.9	73.56±0.47	72.42±0.48	41.83±0.30	41.71±0.36	11.26±0.14
	17.5	73.05±0.47	71.97±0.48	42.21±0.30	40.96±0.36	11.26±0.14
61						
	11.9	73.42±0.47	72.43±0.48	42.22±0.30	41.24±0.36	11.37±0.14
	17.5	73.07±0.47	71.96±0.48	41.57±0.30	41.28±0.36	11.27±0.14
Total						
	11.9	73.49±0.27	72.40±0.28	41.88±0.17	41.48±0.21	11.29±0.08
	17.5	73.01±0.27	71.88±0.28	41.96±0.17	40.98±0.21	11.26±0.08
32		73.18±0.33	72.02±0.34	41.86±0.21	41.10±0.25	11.26±0.10
48		73.31±0.33	72.19±0.34	42.02±0.21	41.33±0.25	11.26±0.10
61		73.25±0.33	72.20±0.34	41.90±0.21	41.26±0.25	11.32±0.10
Two way ANOVA (p)						
SD		0.217	0.180	0.741	0.087	0.789
BA		0.965	0.919	0.853	0.795	0.886
SD×BA		0.971	0.974	0.104	0.425	0.892

Values are mean±SEM.

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction. BW = Body weight, CC = Cold carcass.

with stocking density at or exceeding 30 b/m^2, there was a greater decrease in final BW compared with stocking densities at 10 and 20 b/m^2. However Puron et al. (1995) found no differences in final BW (1.9 kg) and cumulative feed conversion as stocking density increased from 11 to 15 b/m^2. These differences may be due to the using very closely density groups in their study and their broilers had lower final weight than our study.

Marin et al. (1999) showed that chicks escaping in less than 40 s might be more likely to utilize resources for coping with other environmental stressors or for growth. In this study T-maze performance was not affected by breeder age at 3 days of age. The same results between stocking density groups should be due to the not crowding at 3 days

Table 4. Effects of breeder age and stocking density on percentages of abdominal fat and organ weights of broilers

BA (wk)	SD (b/m^2)	Abdominal fat (% BW)	Heart (% BW)	Gizzard (% BW)	Liver (% BW)	Spleen (% BW)	Bursa of fabricius (% BW)
32							
	11.9	1.04±0.07	0.58±0.02	1.47±0.04	1.89±0.05	0.13±0.01	0.20±0.01
	17.5	1.24±0.07	0.63±0.02	1.49±0.04	1.99±0.05	0.13±0.01	0.21±0.01
48							
	11.9	1.58±0.07	0.54±0.02	1.37±0.04	1.88±0.05	0.12±0.01	0.20±0.01
	17.5	1.46±0.07	0.62±0.02	1.41±0.04	1.88±0.05	0.12±0.01	0.20±0.01
61							
	11.9	1.63±0.07	0.58±0.02	1.32±0.04	1.93±0.05	0.12±0.01	0.20±0.01
	17.5	1.69±0.07	0.64±0.02	1.32±0.04	2.01±0.05	0.12±0.01	0.20±0.01
Total							
	11.9	1.41±0.04	0.56±0.01^x	1.39±0.03	1.90±0.03	0.12±0.00	0.20±0.01
	17.5	1.46±0.04	0.63±0.01^y	1.41±0.03	1.96±0.03	0.13±0.00	0.20±0.01
32		1.14±0.05^c	0.61±0.12	1.48±0.03^a	1.94±0.03	0.13±0.01	0.21±0.01
48		1.52±0.05^b	0.58±0.12	1.39±0.03^b	1.88±0.03	0.12±0.01	0.20±0.01
61		1.66±0.05^a	0.61±0.12	1.33±0.03^b	1.97±0.03	0.12±0.01	0.20±0.01
Two way ANOVA (p)							
SD		0.358	0.000	0.572	0.132	0.598	0.902
BA		0.000	0.147	0.002	0.170	0.594	0.946
SD×BA		0.065	0.726	0.855	0.532	0.963	0.965

Values are mean±SEM. $^{a, b, c, x, y}$ Means within columns with different letters are significantly different (p<0.05).

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction, BW = Body weight.

Table 5. Effects of breeder age and stocking density on relative asymmetries (RA), mean relative asymmetry, T-maze performance and tonic immobility reactions (TI) of broilers

BA (wk)	SD (b/m^2)	RA of metatarsus length	RA of metatarsus thickness	RA of tarsometatarsus length	RA of tarsometatarsus thickness	Mean RA	T-maze performance (s)	TI (s)
32								
	11.9	2.48±0.19	5.30±0.61	0.96±0.13	3.62±0.40	3.09±0.20	37.76±2.84	336±22
	17.5	2.13±0.19	4.23±0.61	0.83±0.13	3.85±0.40	2.76±0.20	37.86±2.84	545±22
48								
	11.9	2.21±0.19	4.61±0.61	1.03±0.13	3.14±0.40	2.75±0.20	32.84±2.84	325±22
	17.5	2.11±0.19	5.22±0.61	0.94±0.13	4.00±0.40	3.07±0.20	33.46±2.84	456±22
61								
	11.9	1.87±0.19	5.14±0.61	1.01±0.13	3.74±0.40	2.94±0.20	35.42±2.84	253±22
	17.5	2.43±0.19	4.58±0.61	0.96±0.13	3.40±0.40	2.84±0.20	36.64±2.84	443±22
Total								
	11.9	2.18±0.11	5.01±0.35	1.00±0.07	3.50±0.23	2.92±0.11	35.34±1.64	305±13^x
	17.5	2.23±0.11	4.68±0.35	0.91±0.07	3.75±0.23	2.89±0.11	35.99±1.64	481±13^y
32		2.31±0.14	4.76±0.43	0.89±0.09	3.74±0.28	2.92±0.14	37.81±2.01	440±15^a
48		2.16±0.14	4.92±0.43	0.99±0.09	3.57±0.28	2.91±0.14	33.15±2.01	391±15^b
61		2.15±0.14	4.86±0.43	0.99±0.09	3.57±0.28	2.89±0.14	36.03±2.01	348±15^b
Two way ANOVA (p)								
SD		0.786	0.500	0.376	0.441	0.839	0.781	0.000
BA		0.664	0.968	0.699	0.889	0.986	0.257	0.000
SD×BA		0.059	0.367	0.939	0.320	0.257	0.981	0.159

Values are mean±SEM. $^{a, b, x, y}$ Means within columns with different letters are significantly different (p<0.05).

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction.

of age for chicks.

None of the carcass traits varied significantly due to the different breeder age and stocking density in the present study (Table 3). On the contrary Peebles et al. (1999b) found that percentage of chilled total carcass was significantly higher in broilers from 63 wk old breeders than in those from both 35 and 51 wk old breeders. Similarly, Feddes et al. (2002) showed that stocking density (23.8, 17.9, 14.3 and 11.9 b/m^2) had no effect on breast yield. The percentage of abdominal fat was lower (p<0.001)

Table 6. Effects of breeder age and stocking density on feather conditions, claw length and foot health of broilers

BA (wk)	SD (b/m^2)	Feather condition	Claw length (mm)	Foot health
32				
	11.9	16.4±0.2	11.85±0.13	4.00±0.04
	17.5	14.7±0.2	11.90±0.13	3.80±0.04
48				
	11.9	16.8±0.2	11.96±0.13	4.00±0.04
	17.5	14.9±0.2	11.88±0.13	3.84±0.04
61				
	11.9	16.4±0.2	12.00±0.13	4.00±0.04
	17.5	14.4±0.2	11.70±0.13	3.84±0.04
Total				
	11.9	16.6±0.1^x	11.94±0.08	4.00±0.02^y
	17.5	14.7±0.1^y	11.83±0.08	3.83±0.02^x
32		15.6±0.2	11.87±0.10	3.90±0.03
48		15.9±0.2	11.92±0.10	3.92±0.03
61		15.4±0.2	11.85±0.10	3.92±0.03
Two way ANOVA(p)				
SD		0.000	0.319	0.000
BA		0.099	0.868	0.833
SD×BA		0.896	0.412	0.833

Values are mean±SEM.

Feather condition: 5 (very poor plumage)-20 (intact plumage) for each area of the body neck, breast, back, wings and tail). Foot health: 1 = severely injured matrix and 4 = intact matrix.

^{x,y} Means within columns with different letters are significantly different ($p<0.05$).

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction.

and the percentage of gizzard ($p<0.01$) was higher in broilers from 32 wk old breeders than those of others (Table 4). However percentages of abdominal fat and gizzard were not affected by stocking density in the study. Similarly, Dozier et al. (2006) reported that the amount of abdominal fat was not affected by stocking density. The percentage of heart was higher of broilers at high stocking density in this study. Because litter of pen with 17.5 b/m^2 was worse than that of pen with 11.9 was b/m^2 due to the crowding, these broilers may be having rapid respiration and therefore higher heart percentage. Also McGovern et al. (1999) concluded that heart weight, as a percentage of BW was significantly higher in the feed-restricted broilers. Liver, spleen and bursa of Fabricius are used for anatomical indicators of stress (Freire et al., 2003), but in the present study these indicators were not statistically significant in groups.

Developmental stability may also serve as a noninvasive means for assessing the degree to which an individual is able to buffer its development when stressed (Moller, 1998). Genetic and environmental stressors such as rearing density and lighting regimes (Moller et al., 1999) may increase asymmetry of bilateral traits in poultry. Breeder age and stocking density did not influence the RA of examined parameters (Table 5). Similarly Yalçın et al. (2005) reported that parental age (28 and 58 wk-old age) had no effect on RA of shank length and shank width at 49 d.

Duration of TI has been used as a measurement for evaluating fearful condition and may be used as criteria for measuring well being and levels of stress of chickens (Jones, 1986; Onbaşılar et al., 2007). Broilers from young breeders and broilers stocking high density showed prolonged TI duration ($p<0.001$) therefore these chickens more fearful than the others.

Skin injures of five individual parts of the body were not affected by breeder age and stocking density (data not shown). Feather conditions, claw length and foot health were not affected by breeder age (Table 6). Stocking density influenced the feather condition and foot health ($p<0.001$). These may be due to the worse litter conditions of pens with 17.5 b/m^2 than that of with 11.9 b/m^2.

H-L ratio, serum glucose, cholesterol and triglyceride levels are also as indicators of stress. In this study only serum cholesterol level was affected by breeder age and H-L ratio, serum glucose and cholesterol levels were affected by stocking density (Table 7). Cholesterol level was lower of broilers from young breeders than others ($p<0.01$). H-L ratio, serum glucose and cholesterol levels were higher of broilers reared high stocking density ($p<0.001$). Similarly Freeman et al. (1984) showed that cholesterol level was increased under stress conditions. Puvadolpirod and Thaxton (2000) showed that H-L ratio exhibited a 75% increase at the onset of stress. İpek and Şahan (2006) reported that serum glucose level was not affected by cold stress. This difference among studies may be due to the using different genotypes and different stress factors in experiments.

Humoral immunity, or the ability to produce on antibody response, is commonly employed method of assessing stress. The response to a foreign antigen in an individual depends on several factors such as genetic background, dosage of antigen, route of administration. In this study the antibody titers against SRBC and NDV were not affected by breeder age and stocking density (11.9 and 17.5 b/m^2).

There were no significant interaction between breeder age and stocking density except that feed to gain ratio.

CONCLUSION

As a result of this experiment there were no significant interactions in examined parameters except that feed to gain ratio between breeder age and stocking density. Broilers from 32 wk old breeders had lower initial weight, body weight gain of the first 3 week of rearing, the percentage of abdominal fat and serum cholesterol level; higher

Table 7. Effects of breeder age and stocking density on H-L ratio, serum glucose, cholesterol, triglyceride, anti-SRBC and anti-NDV titers of broilers

BA (wk)	SD (b/m^2)	H-L ratio	Serum glucose level (mg/dl)	Serum cholesterol level (mg/dl)	Serum triglyceride level (mg/dl)	Anti-SRBC titer (5 d) (log_2)	Anti-NDV titer (log_2)
32							
	11.9	0.87±0.06	180±4	75±3	76±2	5.2±0.2	8.9±0.3
	17.5	1.32±0.06	198±4	93±3	81±2	5.3±0.2	9.4±0.3
48							
	11.9	0.81±0.06	189±4	88±3	78±2	5.6±0.2	9.4±0.3
	17.5	1.40±0.06	201±4	92±3	79±2	5.7±0.2	9.4±0.3
61							
	11.9	1.01±0.06	189±4	92±3	78±2	5.7±0.2	9.6±0.3
	17.5	1.38±0.06	198±4	95±3	80±2	5.5±0.2	8.8±0.3
Total							
	11.9	0.90±0.04[x]	186±2[x]	85±2[x]	77±1	5.5±0.1	9.3±0.2
	17.5	1.37±0.04[y]	199±2[y]	93±2[y]	80±1	5.5±0.1	9.2±0.2
32		1.09±0.05	189±3	84±2[b]	79±2	5.2±0.2	9.1±0.2
48		1.10±0.05	195±3	90±2[a]	78±2	5.7±0.2	9.4±0.2
61		1.19±0.05	193±3	93±2[a]	79±2	5.7±0.2	9.2±0.2
Two way ANOVA (p)							
SD		0.000	0.000	0.000	0.127	0.918	0.727
BA		0.229	0.317	0.003	0.932	0.145	0.635
SD×BA		0.223	0.461	0.017	0.592	0.789	0.141

Values are mean±SEM. [a, b, x, y] Means within columns with different letters are significantly different ($p<0.05$).

BA = Breeder age, SD = Stocking density, SD×BA = Stocking density by breeder age interaction.

percentage of gizzard and longer TI duration than those from 48 and 61 wk old breeders. Broilers reared at 17.5 b/m^2, had lower final BW, body weight gain, feed consumption, feather condition, higher percentage of heart, H-L ratio, serum glucose and cholesterol levels, and longer TI durations.

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