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Effect of cage space allowance on egg production, egg quality, immune responses and anti-oxidant variables in White Leghorn layers

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ABSTRACT

A comprehensive study was conducted to optimise cage space allowance (CSA) for White Leghorn (WL) layers (65-80 weeks of age). For the purpose, a total of 900 layers were housed in California colony cages in an open sided poultry house at three different CSA (422, 563, 844 cm^2/bird). Each of the CSA was studied with 20 replicates. The daily egg production (EP), feed intake (FI), feed efficiency (FE, FI/egg mass) and egg quality traits were recorded at the end of each 28d interval. Anti-oxidant responses like lipid peroxidation (LP) and glutathione peroxidase, glutathione reductase and superoxide dismutase in blood were measured at 80 weeks of age. Similarly, the antibody titres against ND vaccine and cell mediated immune response (CMI) against PHA- P were measured at 80 weeks of age. The variation in CSA did not influence EP, egg mass, weight gain and egg shell thickness. The FI reduced and FE improved with reduction in CSA. However, the egg density and egg shell percentage increased with increase in CSA. The LP was higher and the activities of anti-oxidant enzymes were lower at 422 cm^2/bird as compared to the higher space allowances. No effect of CSA on ND titre was observed, while the CMI response was higher at 844 cm^2/bird than the other two CSA. It could be concluded that, though the FE was better at 422 cm^2/b, the trends of shell quality traits and antioxidant variables indicated 546 cm^2/bird as requirement for WL layers in open sided poultry house under tropical regions.

Keywords: Anti-oxidant variables, Cage space allowance, Egg production, Immunity, Laying chicken

Rearing of egg laying chicken in cage system has been highly objectionable for several animal welfare groups across the world. Aviary and furnished cage housing systems have gained wider acceptability as alternatives to cage housing for meeting the welfare and physiological needs of high yielding laying chicken. The furnished cages have the provision for nesting, perching and scratching, which helps in skeletal development and improved bone strength compared to the conventional cages (Jendral *et al.* 2008). However, even now in many developing and developed nations, laying chickens are housed in cage system from day one to the end of egg production cycle with different cage space allowances (CSA) depending on the age and strain of the bird. Optimum CSA is one of the critical/essential components of the bird management, which influences the production potential, bird well-being, stress and immune responses in egg laying strains. Lower CSA (high density) is known to increase oxidative stress and reduce immune responses, and egg production (EP) potential in the modern egg laying chicken (Widowski *et al.* 2017). Limited studies were carried out to find out the effect of CSA for laying chicken in the tropical countries, where the birds are typically reared in open sided poultry house. Therefore, an experiment was conducted to study the effect of three different cage space densities on egg production, egg quality attributes, immune and anti-oxidant responses during post peak production phase (65 to 80 weeks of age) in WL layers.

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MATERIALS AND METHODS

A total of 900 White Leghorn layers (BV 300) of 65 weeks of age were housed at three different cage densities (844, 563 and 422 cm^2/bird) in California type layer colony cages in an open sided poultry house. Each of the space allowances was allotted to 20 replicates and the experiment was conducted from 65 to 80 weeks of age. A standard maize-soybean meal based diet (Table 1) with the recommended levels of nutrients was fed to all the groups. The experiment protocol was carried out as per the guidelines approved by the Institute Animal Ethics Committee (IAEC/DPR/17/1).

The records on egg production (EP), feed intake (FI), feed efficiency (FE, FI/egg and FI / egg mass), egg weight (EW) and egg mass (EM) were measured at weekly intervals. The average EW was recorded by weighing 5 randomly selected eggs per replicate during the last day of each week. The EM was calculated by multiplying the average EW with the total number of eggs produced in each

Table 1. Ingredient and nutrient composition of layer diet

Ingredient	g/100 g
Maize	62.6
Soybean meal 45%	11.8
Distillery dried grain with solubles	8.00
Mustard cake extraction	5.00
Mono calcium phosphate	0.39
Phytase 5000	0.01
Stone grit	8.40
Lime stone powder	3.00
Salt	0.44
DL-Methionine	0.08
L-Lysine HCl	0.00
L-Threonine	0.02
Premix[1]	0.30
Nutrient composition	
Metabolizable energy (kcal/kg)[2]	2601
Protein (%)[3]	16.00
Lysine (%)[4]	0.795
Methionine (%)[4]	0.330
Calcium (%)[3]	3.800
Available phosphorus (%)[4]	0.330
Sodium (%)[4]	0.190
Threonine (%)[4]	0.600

[1]Provided (mg/kg diet): thiamin 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 15; pantothenic acid, 10; α tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2; retinol acetate, 2.75; cholecalciferol, 0.06; choline, 650; copper, 8; iron, 45; manganese, 80; zinc, 60; selenium, 0.18; hydrated sodium calcium alumino silicates, 800; phytase, 375 units. [2] Calculated; [3]Analyzed; [4]Calculated based on analyzed ingredient composition.

replicate and expressed as g per hen per day. Individual body weight (BW) of the birds was recorded on all the birds at the beginning (65 weeks of age) and at the end of the experiment (80 weeks of age) and the difference was expressed as body weight gain (BWG).

The egg quality parameters were recorded by collecting 3 eggs from each replicate at the end of each 28d period. Egg quality was determined in terms of density, shell breaking strength, shell weight, shell thickness and Haugh unit (HU) score (Egg Multi Tester, EMT- 5200, Robotmation Co. Ltd., Tokyo, Japan). Egg shell thickness was measured with digital thickness gauge (Mitutoyo Code 7027, Mitutoyo Corporation, Kanagawa, Japan) at the broad end, equatorial region and narrow end and the mean was taken as the shell thickness. Egg shell breaking strength was measured using an universal testing machine (EZ Test, Shimadzu, Japan) with a 50 N load cell at a speed of 5 mm/minute.

The LP activity in the serum was expressed in nmol MDA/mg protein (Placer *et al.* 1966). The activities of GSHPx, GSHRx and SOD were estimated by following methods described by Paglia and Valantine (1967), Ethelbert *et al.* (2012) and Azad *et al.* (2017), respectively.

The effect of CSA on cell mediated immunity (CMI) and HI response against Newcastle disease vaccination was studied. The blood samples were collected at 80 weeks of age from one bird per replicate. The antibody titres in sera against ND virus were measured by haemagglutination test (Reynolds and Maraqa 2000). The CMI response was assayed by cutaneous basophilic hypersensitivity test *in vivo* by using phytohemaglutinin-P (PHA-P) by using the method described by Corrier and Deloach (1990).

Statistical analysis: Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The variations in the different parameters were analysed using the one-way analysis of variance procedure, wherein different CSA were designated as the fixed factors and response variables were taken as the dependent variables. The statistical model of variance analysis used is as follows:

$$Yij = \mu + Ti + eij$$

where, μ, overall mean; Ti, fixed effect of cage space; eij, random error present in the j-th observation on the i-th treatment. On the detection of overall significant difference, the means of different treatments were separated using Tukey's test.

RESULTS AND DISCUSSION

Variation in CSA did not influence ($P>0.05$) the EP, BWG, EW and EM, whereas the daily FI and FE (FI/egg and FI/EM) were significantly ($P<0.05$) affected with the variation in CSA (Table 2). The FI increased progressively and significantly ($P<0.05$) with increase in space allowance. The highest FI was observed in layers housed with 844 cm^2 floor space followed by 546 and 422 cm^2/bird in the order. The amount of feed required to produce an egg (FI/egg) or unit EM (FI/EM) was significantly ($P<0.05$) higher in layers housed at the highest cage space (844 cm^2/b) compared to those reared at 422 cm^2/b. The FE in 546 cm^2/b group was intermediate. However, several reports (Carey *et al.* 1995, Rios *et al.* 2009) also did not observe any effect of cage space on EP. However, few authors (Cunningham *et al.* 1988) reported significant reduction in EP when the cage space was reduced (406 to 316 cm^2/bird). The variation in EP responses among different studies could be due to the different range of space allowances tested in different studies. The lowest CSA tested in the present study and those of Lee (1989) and Carey *et al.* (1995) were probably did not adversely affect the performance of birds. The reduced feeder space per bird due to severe restriction in cage space was attributed as the cause of reduced FI and the corresponding reduction in EP (Okpokho *et al.* 1987). It appears that the WL layers need approximately 422 cm^2/b cage space without affecting the EP and the space lower than that showed negative response as observed by Okpokho *et al.* (1987) and Cunningham *et al.* (1988). Cunningham *et al.* (1988) reported 2.1% reduction in EP when cage space was reduced from 406 to 316 cm^2/ bird. Few authors studied higher space allowance (450, 562 or 1,125 cm^2/ bird) than the levels tested in the current study and did not find any difference in EP or EW (Rios *et al.* 2009). The negative effects of cage space on EP and other performance

Table 2. Effect of different cage space allowances (CSA) on egg production performance of WL layers (65 to 80 weeks)

CSA (cm^2/b)	EP (%)	FI/bird (g)	FI/egg (g)	FI/EM (g)	BWG (g)	EW (g)	EM (g/d)
422	86.73	107.0^{c}	123.7^{b}	2.068^{b}	222.2	59.85	51.92
546	87.29	108.7^{b}	124.9^{ab}	2.096^{ab}	167.3	59.65	52.08
844	87.51	110.6^{a}	127.0^{a}	2.131^{a}	170.5	59.66	52.18
SEM	0.41	0.19	0.61	0.01	18.4	0.12	0.23
N	20	20	20	20	20	20	20
P value	0.72	0.001	0.089	0.026	0.40	0.72	0.90

CSA, cage space allowance; EP, egg production; FI, feed intake; EM; egg mass; BW; body weight; EW, egg weight; BW, body weight; EM, egg mass. $^{a\,b}$Means having common superscripts in a column differ significantly (P<0.05).

variables was evident in other studies (Anderson *et al.* 2004, Sarica *et al.* 2008), where heavy egg laying strains (Dekalb XL, ISA Brown, Hy-Line Brown) were used, whose body weight was higher than the WL layers strains. Anderson *et al.* (2004) reported significant reduction in EP from 82.3% to 77.4% with reduction in cage floor space from 482 to 361 cm^2/b in Hy-Line W36 and egg laying strains. Similarly, Sarica *et al.* (2008) reported significant drop in EP with reduced stocking densities (1968/2000 to 393.8/500 cm^2 per hen, respectively) in brown egg layers (Hyline Brown and ISA brown, respectively), who reported body weight was more than 2000 g at the end of the experiment. However, significant reduction in EP was reported by Saki *et al.* (2012) in WL layers during peak production phase (35 to 47 weeks of age) at 500 or 667 cm^2/b compared to those reared at 1000 or 2000 cm^2/b.

The BWG was not affected with the variation in CSA in the current study. Contrarily, significant increase in live weights of layers at higher cage space was observed by Sarica *et al.* (2008), who provided much higher cage space (1000 and 2000 cm^2/bird) than in the current study. The proportionate increase in feeder space per bird with the increase in cage space was reported to increase the FI and body weight (Cunningham and Ostrander, 1982, Ouart and Adams 1982). The daily EM was not affected in the current study, while Jahanian and Mirfendereski (2015) reported significant reduction in EM with increase in stocking density. Though the stocking density used by Saki *et al.* (2012) was nearer to the current study (422 vs 500 cm^2/b), the EP and EW were significantly reduced in their study due to the heavy body weight of layer strain used, which reduced the EM, while such reduction in performance was not observed in the current study.

The reduced performance reported (Adams and Craig, 1985, Davami *et al.* 1987, Okpokho *et al.* 1987) in layers reared at lower cage space allowance was attributed to the reduced feeder space and the consequent reduction in FI. Though the FI was significantly reduced in the current study at lower CSA (422 cm^2/b), the average FI was 107 g/b/d with the calculated daily intake of 278 kcal ME and 17.1 g/b/d protein, respectively which are adequate for the strain at the age as observed in our previous studies (Rama Rao *et al.* 2011, 2014).

Similarly, Hill (1977) also reported improvement in FE at higher cage density (310 cm^2/bird) compared to those reared at 464 cm^2/bird. The birds housed at lower CSA might have had lower activity and proportionately lower energy expenditure for activity compared to those reared at the higher space allowance. This assumption has the support of Rhim and Jae (2014), who reported that total time spent for activity (walking and standing) by egg laying hens was higher in large cage space (940 cm^2/b) compared to the small cage space (420 cm^2/b). The progressive increase in FI with increase in the CSA observed in the current study also suggest the increased requirement of the nutrients for birds having higher space allowances, probably for meeting the increased physical activity. Similar to the current observations, Rios *et al.* (2009) also reported decreased FI with increased bird density. This might be due to the lower movement / activity of birds in denser environment (422 cm^2/b) compared to those, which got more floor space for activity and movement. Similar to our results, significant improvement in FE was reported (Saki *et al.* 2012) at the higher stocking densities in White Leghorn hens, which was attributed to the limited movement of the birds at lower space allowance.

The improved FE at lower CSA in the current study contradicts the findings of Adams and Craig (1985), Davami *et al.* (1987) and Rios *et al.* (2009), who observed improved feed efficiency (per dozen or per EM) as the available cage space increased. In the current study, the FI reduced with reduction in CSA without affecting the EP and therefore, the FE was higher at the lower CSA compared to those reared with higher floor space.

Egg weight was not affected by the CSA in the current study. Similarly, Carey *et al.* (1995) and Rios *et al.* (2009)

Table 3. Effect of different cage space allowances (CSA) on egg quality in WL layers (65 to 80 weeks)

CSA (cm^2/b)	Density	Strength (N)	HU	SW (g)	ST (mm)	Shell (%)
422	1.069^{b}	13.65	66.74	5.139	0.367	8.215^{b}
546	1.071^{a}	14.13	66.82	5.207	0.362	8.460^{a}
844	1.070^{ab}	13.50	66.24	5.213	0.361	8.467^{a}
SEM	0.01	0.37	0.51	0.04	0.01	0.05
N	20	20	20	20	20	20
P value	0.03	0.72	0.88	0.64	0.14	0.09

CSA, cage space allowance; HU, Haugh unit; SW, shell weight; ST, shell thickness. $^{a\,b}$Means having common superscripts in a column differ significantly (P<0.05).

Table 4. Effect of different cage space allowances (CSA) on anti-oxidant and immune responses in WL layers (65 to 80 weeks)

CSA (cm^2/b)	LP (nmol MDA/ mg protein)	GSHPx	GSHRx	SOD	HI (Log_2)	CMI (%)
		(Units/ml)				
422	3.668^a	849^b	941^b	4.990^b	6.825	104.5^b
546	3.602^{ab}	1032^{ab}	1159^a	4.960^b	6.700	102.3^b
844	3.263^b	1095^a	1181^a	5.844^a	6.850	119.1^a
SEM	0.07	41.77	43.3	0.14	0.09	4.24
N	20	20	20	20	20	20
P value	0.046	0.042	0.041	0.015	0.77	0.02

CSA, cage space allowance; LP, lipid peroxidation; GSHPx, glutathione peroxidise; GSHRx, glutathione reductase; SOD, superoxide dismutase; HI, haemagglutination inhibition; CMI, cell mediated immune response. $^{a\,b}$Mean having common superscripts in a column differ significantly (P<0.05).

also reported lack of cage space effect on EW. Contrary to the above results, significant reduction in EW was reported in the literature (Davami *et al.* 1987, Rios *et al.* 2009) with increase in cage density. The primary reason for such reduction in EW could be due to the magnitude of cage space reduction (321 and 300 cm^2/b) in their experiments, which was considerably lower than the lowest cage space given in the current study (422 cm^2/b). The cage space in the above studies was less by about 24 and 26.5%, which might have proportionately reduced the availability of feeder space and eventually the feed consumption.

The egg density was significantly higher (P<0.05) in the groups housed at 546 cm^2/b compared to those reared at 422 cm^2/b (Table 3). The egg density at the highest floor space (844 cm^2/b) was intermediate between the above two groups. The CSA did not influence (P>0.05) egg breaking strength, HU score, shell weight and shell thickness, while the shell percent showed a trend of improvement (P<0.10) at higher CSA (546 and 844 cm^2/b) compared to those reared at the lowest CSA tested (422 cm^2/b). Similar to our findings, Rios *et al.* (2009) and Campbell *et al.* (2017) reported that the egg quality parameters were not affected with variation in cage density. However, the egg density and shell percent showed a trend of improvement with increase in cage space in the current study. The exact reason for improvement in these egg quality variables is not known, however, the increased activity of birds at higher space allowance might have increased mineral metabolism, which might have improved the egg density and shell per cent in layers reared at higher floor space allowance.

The LP was significantly (P<0.05) higher at the lowest CSA (422 cm^2/bird) compared to those reared at 844 cm^2/bird and was intermediate in 546 cm^2/bird group (Table 4). On the other hand, the activities of anti-oxidant enzymes (GSHPx, GSHRx and SOD) in serum increased (P<0.05) with the increase in CSA. The HI titre against ND vaccine was not affected (P>0.05) with the variation in CSA (Table 4). The CMI response to PHA-P inoculation was significantly higher (P<0.05) at the highest CSA (844 cm^2/b) compared to the other two space allowances. Similarly, Tactacan *et al.* (2009) also reported no significant difference in antibody response to ND vaccine in layers reared at different cage house environment and space allowance. The CMI response to PHA-P was significantly higher in layers reared at the highest space allowance (844 cm^2/b) compared to the other levels of CSA.

During stress conditions, several reactive oxygen species are produced at much higher concentration, which causes cell damage due to lipid oxidation. The MDA is the end product of lipid oxidation and is an important indicator of stress (Gawel *et al.* 2004). The magnitude of LP was measured as MDA concentration in serum. In the current study, the LP was higher and the concentrations of anti-oxidant enzymes were reduced in layers reared at lower CSA, which suggests that the birds reared at the lowest CSA were under more oxidative stress (Simsek *et al.* 2013) compared to those reared at the higher CSA.

Based on the data, it can be concluded that the feed efficiency was significantly higher in White Leghorn layers reared at 422 cm^2/b compared to the higher cage space allowance. However, for better egg quality (shell percent and egg density), cell mediated immunity and anti-oxidant responses (reduction in lipid peroxidation and higher activities of GSHPx, GSHRx and SOD), the layers need 546 cm^2/b cage space than the optimum space for the feed efficiency (422 cm^2/b).

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