Pakistan J. Zool., pp 1-8, 2022. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220701050728

Effect of Cage and Floor Rearing System on the Reproductive Performance and Immunocystochemistry of Pituitary Cells of Broiler Breeders

Waqas Alam[1], Sar Zamin Khan[1] and Rifat Ullah Khan[2]*

[1]Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan
[2]College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

Article Information
Received 01 July 2022
Revised 15 June 2022
Accepted 20 July 2022
Available online 15 September 2022 (early access)

Authors' Contribution
SAK and WA presented the concept of the study, performed the experiments and wrote the manuscript. SAK and RUK supervised the study and edited the manuscript. WA performed the data analyses. RUK helped in editing the manuscript. WA helped in the analysis with constructive discussions. WA did the formal data analysis and raw materials supply.

Key words
Cage housing system, Floor housing system, Egg quality and quantity, Fertility and hatchability

ABSTRACT

A total of 200 broiler breeders (22 weeks old) having uniform body weight were selected and randomly distributed into cage (60.96 × 60.96 × 53.34 cm) and floor housing systems having 90 females and 10 males. Each group was subdivided into three replicates (30/replicate female). Hubbard management guideline was followed for feeding, watering, and vaccination. Egg quality parameters were evaluated at pre-peak (22-30 weeks), peak (31-40 weeks) and post-peak (41-59 weeks) production stages. Compare to floor housing system, age of hen at first lay and mortality were lower ($P \leq 0.05$) in cage housed birds. Egg production percentage and feed efficiency were significantly ($P<0.05$) higher in cage birds compared to the floor. Fertility and hatchability percentage were lower ($P<0.05$) in pre-peak stage and then increased during peak and post-peak stages. There was no significant effect of the housing system on the immunocytochemistry of the cells of follicle stimulating hormone (FSH), leutenizing hormone (LH) and growth hormone (GH). The results of the present study indicated that birds kept under cage had enhanced egg production, fertility, hatchability and lower mortality than the birds managed on floor.

INTRODUCTION

An external factor that affects productive features and egg quality is the laying hen raising system, which has been the subject of extensive scientific research (Dong *et al*. 2017). According to some researchers (Rossi, 2007; Hidalgo *et al*., 2008; Dong *et al*., 2017), cage-raised hens produced more eggs and had better egg quality attributes than hens raised in other settings. The hormonal health of the hen controls how many eggs are produced (Pirsaraei *et al*., 2008). Changes in reproductive hormones such luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (P4), and prolactin (PRL) may result in various performance qualities in layers (Onagbesan *et al*., 2006; Li *et al*., 2011).

Housing birds in cages or on floors can have both positive and bad effects on their well-being, growth, and reproduction. Birds have traditionally been raised in floor-rearing methods ever since they were domesticated. As egg production technology advanced, traditional cages increasingly took over as the primary housing option. Battery cages are criticised due to concerns about animal welfare because they reduce bird output by stressing them out and limiting their ability to exhibit their natural behaviour. However, maintaining a clean environment lowers the risk of sickness (Al-Bahouh *et al*., 2012). The poultry business suffers from substantial welfare problems due to the deprivation of natural activities in cages (Shields and Duncan, 2009). A cage housing system eliminates the need for a nest because it has self-nesting room and saves farmers money. In the current study, it was expected that the various housing arrangements (cage vs. floor) may have a significant impact on the productivity of broiler breeders.

In the conventional way of rearing, birds are raised on the ground and brooded before being relocated to cages when egg production starts. Most broiler breeders

* Corresponding author: rukhan@aup.edu.pk
0030-9923/2022/0001-0001 $ 9.00/0

are kept on the deep floor rearing system in developing nations, including Pakistan, where fertile hatching eggs are produced. Notably, the floor raising system is a popular and affordable method of rearing (Aviagen, 2013). However, birds kept indoors are more likely to contract infections (Yaniz *et al*., 2010). Currently, millions of birds are raised in cages under an ecologically controlled system instead of the traditional housing system (Habibullah *et al*., 2016). Therefore, the most effective housing system for producing profitable poultry was determined by comparing its reproductive and productive characteristics. The aim of the present study was to investigate the cage and floor housing system on the productive and reproductive outcome of broilers from 22 to 50 weeks of age.

MATERIALS AND METHODS

Ethical considerations and study site

This research study was pre-approved by the Departmental Board of Studies meetings on ethics, methodology and welfare of birds, KP Agriculture University Peshawar, Pakistan.

Experimental design and bird's selection

A total of 200 days old female and 50 days old male broiler breeders were placed in the poultry shed using the practices recommended by poultry companies. In floor housing system, 90 female broiler breeders were raised on standard space (3 × 3 feet). Males were added in the ratio of 1: 10 to both housing systems. In cage system, 90 females were reared in each individual cage (60.96 × 60.96 × 53.34 cm).

For the first 20 weeks of life, the males were housed in two identical pans and the females in six similar pens. The chicks received unlimited access to water during the whole experiment. Throughout the rearing and production phases, nibble drinkers were employed. Feed restriction was introduced following the second week. Every day, feed was delivered. 10 chicks from each pen were randomly selected at 2 weeks of age. They were reared utilizing the skip-a-day strategy of feed limitation from 12 to 19 weeks. Males and females birds were equally divided into four floor pens or four two-tier cages two weeks before the start of egg production (20 weeks of age). The broiler breeder management manual's instructions for restricted feeding. The stocking density of floor pen and two cages unit occupied the same space.

Initially chicks were reared at a temperature of 90°F, which was gradually decreased at the rate of 5°F per week until a temperature of 70°F was reached. For the first three days, artificial light was provided for twenty-four h every day. From day 4 through day 21, the birds received 12 h of light every day, they were 20 weeks old. In all age groups, water was freely available via nipple drinkers.

Egg collection

Eggs were gathered 3 times each day during pre-peak (22-30 wks), peak (31-40 wks) and post peak (41-50 wks). Fertility test was held each week on ten eggs per replicate.

Reproductive performance

The reproductive traits such as age and weight of hen at first lay, weight of first lay egg and peak egg production was recorded. Mortality of the birds was recorded on daily basis when occurred.

Egg production and feed efficiency

Daily egg production was recorded. Feed efficiency was calculated on the basis of feed required for dozen of egg production or feed required for 1 kg egg production.

Egg fertility and hatchability

Eggs were incubated for 10 days after which they were broken out to test the fertility of the eggs. Egg samples were delivered to the lab after being kept for 24 h at 55°F and 65% humidity. Egg fertility and hatchability were determined as follow:

Egg fertility = fertile eggs ÷ number of eggs set × 100

Egg hatchability= hatched eggs/ total fertile eggs set × 100

Removal of pituitary gland and slides preparation

At the end of peak production, five birds per replicate were slaughtered. Skin and feathers were removed around the head. The pituitary gland was removed from below the brain mass. Pituitary gland was immediately immersed in Bouin's Holland solution for 24 h following treatment of 4% formaldehyde and then embedded in paraffin wax. The samples were cut into slices of 4µm thin sections with the help of microtome (Microm GmbH, Walldorf, Germany). The samples were placed on slides precoated with 0.1% poly-L-lysine.

Immunohistochemistry of FSH, LH and GH cells

The following steps were used to localize antigens of FSH, LH and GH in pituitary gland samples by using specific antibodies (Khan *et al*., 2013). Tissue sample were deparaffinized and rehydrated. Antigen retrieval/ unmasking of the antigen were done in Tris HCl solution (0.1 M: pH 6.6) at 121°C for 10 min. The H_2O_2 was applied for washing the specimen, and deactivating the peroxidase activities, incubate for 10 min at room temperature. Washing four times with PBS was repeated and add BSA (bovine serum albumin)/ protein block were applied to the sections for 5 min to block the non-specific sites. Before drying, prediluted primary antibody of FSH and LH were

added to the section and keep the slide in a humidified chamber for 2 h at room temperature. Slide was washed four times with PBS, then applied secondary antibody on the tissue sections and incubate for 10 min. These washing and incubation process were repeated after applying streptavidin peroxidase, chromogenic and DAB substrate for 30 min for color development. Normal goat serum (10%) was used on negative control slides.

Morphometric analysis of immunoreactive cells

A compound microscope was used to conduct morphometric analysis. For this analysis, cells with excellent cross sections and clean non-reactive nuclei were chosen. Total six samples per group were considered. Two slides for each sample were prepared and total microscopic fields slides were counted with the help of Image J Software (Image J 1.44P Wayne Rasband, National Institutes of Health, and Bethesda, MD, USA).

Statistical analysis of data

General linear model (GLM) procedure and completely randomized design (CRD) was used to analyze the experimental data. Birds on floor and cage were compared under t-test. Least significant difference (LSD) test was applied for difference in significance at probability level P≤0.05 (SAS, 1996).

RESULTS

Table I shows the effects of different production systems (cage vs. floor) on broiler breeder performance. Broiler breeders raised in cages begin egg production two weeks earlier (P≤0.05) than broiler breeders raised on the floor. The weight of the hen and the weight of the egg at the first lay was not significantly (P≥0.05) different under either housing system. However, in the cage housed system, the peak egg production age was lower (P<0.05) compare to the floor birds. Broiler breeders reared in cages had a higher (P≤0.05) peak egg production percentage and lower mortality than those kept on the floor.

Data on egg production, and feed conversion ratio (FCR) of broiler breeders reared under floor versus cage housing systems are presented in Table II. Overall, egg production at pre-peak, peak and post peak was considerably higher (P≤0.05) in cage housing system compared to floor housing system. Similarly, significantly improved feed efficiency (P≤0.05) was recorded in cages at pre-peak, peak, and post peak production stage than floor housing.

Table III shows the fertility and hatchability of broiler breeders raised on the floor compared in cages. Floor housed broiler breeders had a significantly greater (P≤0.05) pre-peak fertility percentage than cage housed broiler breeders, whereas caged birds had a higher (P≤0.05) hatchability than floor at pre-peak production stage. Cage housing system had greater fertility and hatchability throughout peak and post-peak production stages than floor.

Table I. Experimental diet of broiler breeders.

Ingredients%	Pre peak	Peak	Post peak
Maize corn	61.79	64	62.92
Wheat bran	8.19	5.0	6.36
Soybean meal	18.7	19.2	18.92
Corn gluten meal	2.00	2.00	2.00
Dicalcium phosphate	1.3	1.2	1.2
Limestone	6.2	6.2	6.2
Salt	0.41	0.41	0.41
Lysine-HCL	0.06	0.05	0.05
DL- Methionine	0.03	0.07	0.07
Choline -Cl and Coban90	1.22	1.22	1.22
Vitamin premix[1]	0.1	0.1	0.1
Mineral premix mintrex[2]	0.05	0.05	0.05
Coccdiostat	0.05	0.5	0.5
Total	100	100	100
Calculated analysis (%)			
Crude protein (CP)	15.3	16.04	15.90
ME per kg	2750	2920	2800
Met and cysteine	0.50	0.64	0.58
Lysine	0.62	0.83	0.66
Ca	1.50	3.30	3.39
P	0.41	0.41	0.38
Chemical analysis %			
Crude protein	15.45	16.14	16.02

[1] A, 82,000 IU; D3, 12500 IU; B2, 45 mg; B1, 4 mg; B6, 8 mg; B12, 40 µg; E, 20 mg; niacin, 60 mg
[2] Contained: Ca, 32%; Mn, 0.44%; P, 6%; I, 150 ppm; Zn, 0.33%; Cu, 250 ppm; Fe, 2000 ppm; calcium pantothenate, 12.5 mg

Table II. Reproductive performances of broiler breeders under cage versus floor system.

Parameters	Housing system		P value
	Cage	Floor	
Age of hen at first lay (week)	22^{b}±0.28	24^{a}±0.30	0.00
Weight of hen at first lay (g)	2400.2± 115.47	2341.6± 110.23	0.71
Egg weight at first lay (g)	51.23±0.54	50.51±0.39	0.39
Peak production age (weeks)	30.32^{b}±0.46	32.50^{a}±0.31	0.05
Mortality (%)	3.25^{b}±0.59	7.12^{a}±0.67	0.00

[ab] means with different letters within the rows differs at P < 0.05.

Table III. Egg production and feed efficiency of broiler breeders under floor vs cage rearing system.

Stages of production	Egg production (%)		P value	Feed efficiency (%)		P value
	Cage	Floor		Cage	Floor	
Pre peak (22-30 wks)	49.40±1.30 [a]	44.31±1.10 [b]	0.00	3.19±0.29 [b]	4.00±0.49 [a]	0.01
Peak (31-40 wks)	86.28±0.50 [a]	84.88±1.12 [b]	0.04	2.18±0.45 [b]	2.47±0.89 [a]	0.04
Post peak (41-50wks)	83.49±0.67 [a]	82.28±1.13 [b]	0.05	2.19±0.03 [b]	2.45±0.79 [a]	0.04

[ab] means with different letters within the rows differs at P < 0.05. wks for weeks.

Table IV. Fertility and hatchability of broiler breeders under floor vs cage rearing system.

Stages of production	Fertility (%)		P- Value	Hatchability (%)		P- Value
	Cage	Floor		Cage	Floor	
Pre peak (22-30 wks)	63.99±0.98 [b]	70.78±0.29 [a]	0.001	78.60±0.48 [a]	74.42±0.18 [b]	0.01
Peak (31-40 wks)	93.58±0.22 [a]	90.89±0.19 [b]	0.03	84.55±0.46 [a]	79.63±0.41 [b]	0.001
Post peak (41-50 wks)	93.00±0.09 [a]	90.61±0.05 [b]	0.05	83.99±0.31 [a]	79.16±0.98 [b]	0.001

[ab] means with different letters within the column differs significantly at P < 0.05 Wks for weeks.

Table IV shows the number and size of FSH, LH and GH cell, respectively in microscopic field area of chicken pituitary gland reared under floor and cage housing system. The result showed that the number and size of FSL, LH and GH under both housing system were not disturbed (P≥0.05).

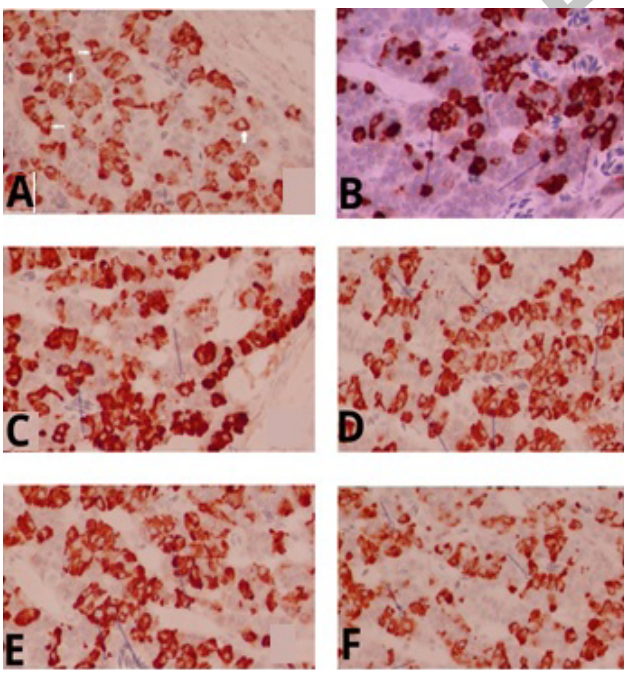

Fig. 1. Immunocytochemistry of FSH cells of cage birds (A), FSH cells of floor (B), GH cells of cage birds (C), GH cells of floor birds (D), LH cells of cage birds (E) and LH cells of floor cells (F) Scale bar = 200 µm.

Table V. Number and mean size (µm) of FSH, LH and GH cells in microscopic field area of pituitary gland of broiler breeders under floor versus cage housing system.

Group	FSH	LH	GH
Number of cells/ microscopic field			
Cage	40.67±5.03	39.33±1.15	43±3.606
Floor	42.67±9.07	38±1.00	44±7.55
P-Value	0.760	0.20	0.85
Size of cells (µm)			
Cage	9.01±0.16	6.67±0.02	7.12±0.04
Floor	9.23±0.03	7.12±0.07	6.98±0.02
P-Value	0.573	0.090	0.375

FSH, follicular stimulating hormone; LH, luteinizing hormone, GH, growth hormone.

DISCUSSION

In the current study, the cage housing arrangement resulted in considerably increased egg production and decreased mortality. These findings are similar with those made by Voslarova *et al*. (2006) and Dong *et al*. (2017). It is commonly accepted that eggs with weak shells, fractured shells, or no shells are more susceptible to breaking when hens peck at them in floored flocks. The cracked egg will be swiftly consumed by hens, going unnoticed (Khan and Khan, 2018). The hostility and injury

of the birds in the competition for food and their innate dominant tendency may be to blame for the greater death rate in the floor housing arrangement. The increased death rate on the floor may be related to exposure to microbial contamination of food, water, and litter. The primary indicator of inadequate welfare is mortality (Blokhuis *et al*., 2007). Due to automation in feeding and drinking, the cage housing used in this study has the lowest likelihood of such contamination and is best suited for the production of high-quality eggs (Stanley *et al*., 2013; Philippe *et al*., 2020).

In the current investigation, the cage system had a much higher feed efficiency than the floor. Dong *et al*. (2017) discovered that Xianju chickens raised in cages had a significantly lower FCR than those raised on the floor. They also believed that because floor-raised birds had more room to peck, walk, run, and engage in natural behaviours, they consumed more energy and experience growth restrictions. These findings are similar to those of Starcevic *et al*. (2021).

In the current study, fertility and hatchability were significantly different in cages than on the floor, supporting the findings of Khabisi *et al*. (2012) and Habibullah *et al*. (2016). According to Penfold *et al*. (2000) and Brillard (2003), there is a trend towards the hatch during the pre-peak production period of egg laying, while Sayyazadeh and Shahsavarani (2005) found that there is an increase in fertility and hatchability during the post-peak production phase. Hatchability increased from the pre-peak to the peak and subsequently decreased with the production phase. Similar trends have been seen by Brillard (2003), Penfold *et al*. (2000), Mahmoud *et al*. (1996), and McDaniel *et al*. (1996) in relation to the low percentile of hatch during the pre-peak period of lay. After reaching a peak, flock fertility naturally declines as hen ages, which is a physiological fact. Male broiler breeders experience physical issues as they mature. As a result, broiler breeders using a floor system had poorer fertility due to both large body weight and advancing age (Bramwell *et al*., 2004). Furthermore, since more birds must eat from a single floor feeder, the rivalry for food is more significant than in a cage. Floored birds' uneven feed consumption reduces flock homogeneity, which causes them to lay eggs of varying sizes and lowers the percentile hatch rate (Dong *et al*., 2017; Khan and Khan, 2018). The eggs generated in deep litter are more susceptible to faecal contamination, where the likelihood of bacterial contamination is greater; this infectious organism may have penetrated the egg and resulted in the decreased fertility and hatchability in floor housing systems.

Hubbard broiler breeders housed in cages lay more eggs there than on the ground. These results are consistent with Hulzeboschs (2006) and Yakabu *et al*. (2007) findings. The same flock was placed on the floor two weeks after the broiler breeders in cages started producing eggs at the Hubbard Company-recommended age. By providing the cages, the productivity of the birds increases (Huneau-Salaun *et al*., 2011). Currently, egg production in cages grew more during pre-peak by 5.09 %, peak by 1.4 %, and post-peak by 1.21 % than in the floor housing system. Due to the comfortable environment in the cages, greater percentage of egg were produced. Tumova and Ebeid (2005) and Pistekova *et al*. (2006) found larger numbers of high-quality eggs in floor housing systems, contradicting the findings of the current study (Ericsson *et al*., 2016).

The number and dimension of immunostained cells in anterior pituitary gland was non-significantly different under both housing system. There is a dearth of information on the immunostained cells of FSH, LH and GH in poultry pituitary gland. The present study provides a baseline of profitable and healthy poultry production in cage rearing system. Li *et al*. (2017) found higher level of FSH, LH hormones in breeders raised in cages.

CONCLUSION

Hubbard broiler breeders reared in cages showed better performance in term of improved fertility, hatchability, egg production and feed conversion ratio compared to floor housing.

ACKNOWLEDGEMENT

This study was financially supported by Higher Education Commission of Pakistan (HEC) under national research program for universities (NRPU) under project number (20-3783/NRPU/R&D/HEC/2014 /690).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Al-Bahouh, M.E., Al-Nasser, A.Y., Abdullah, F.K., Ragheb, G. and Mashaly, M.M., 2012. Production performances of different broiler breeds under different housing system. *Int. J. Poult. Sci.,* **11**: 190–195. https://doi.org/10.3923/ijps.2012.190.195

Aviagen, 2013. *Ross 308 parent stock management handbook*. Aviagen, Newbridge. pp. 70–75.

Blokhuis, H.J., Van Niekerk, T.G.C.M., Bessei, W., Elson, H.A., Guemene, D., Kjaer, J.B., Levrino, G.A.M., Nicol, C.J., Tauson, R.K., Weeks,

C.A. and De-weerd, H.A.V., 2007. The Lay Wel project: welfare implications of changes in production systems for laying hens. *Worlds Poult. Sci. J.*, **63**: 101-114. https://doi.org/10.1017/S0043933907001328

Bramwell, R.K., Mcdaniel, C.D., Wilson, J.L. and Howarth, B., 1996. Age effect of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. *Poult. Sci.*, **75**: 755-762. https://doi.org/10.3382/ps.0750755

Brillard, J.P., 2003. Practical aspects of fertility in poultry. *Worlds Poult. Sci. J.,* **59**: 441-446. https://doi.org/10.1079/WPS20030027

Dong, X.Y., Yin, Z.Z., Ma, Y.Z., Cao, H.Y. and Dong, D.J., 2017. Effects of rearing systems on laying performance, egg quality, and serum biochemistry of Xianju chickens in summer. *Poult. Sci.,* **96**: 3896-3900. https://doi.org/10.3382/ps/pex155

El-Deek, A. and El-Sabrout, K., 2019. Behaviour and meat quality of chicken under different housing systems. *Worlds Poult. Sci. J.,* **75**: 105-114. https://doi.org/10.1017/S0043933918000946

Ericsson, M., Henriksen, R., Belteky, J., Sundman, A.S., Shionoya, K. and Jensen, P., 2016. Long-term and transgenerational effects ofstress experienced during different life phases in chickens (*Gallus gallus*). *PLoS One*, **11**: e0153879. https://doi.org/10.1371/journal.pone.0153879

Ferrante, V., Susanna, L., Giuseppe, V. and Cavalchini, L.G., 2009. Effects of two different rearing systems (organic and barn) on production performance, animal welfare traits and egg quality characteristics in laying hens. *Ital. J. Anim. Sci.,* **8**: 165–174. https://doi.org/10.4081/ijas.2009.165

Ghanem, H.M., Ateya, A.I., Saleh, R.M. and Hussein, M.S., 2017. Artificial insemination vs natural mating and genetic prl/psti locus polymorphism and their effect on different productive and reproductive aspects in duck. *Adv. Anim. Vet. Sci.,* **5**: 179-184.

Habibullah, M., Hashem, M.A., Rana, M.S. and Islam, M.H., 2016. Effect of artificial insemination on different production parameter in Hubbard classic broiler parent stock. *J. Bangladesh Agric. Univ.,* **13**: 71-77. https://doi.org/10.3329/jbau.v13i1.28720

Hidalgo, A., Rossi, M., Clerici, F. and Ratti, S., 2008. A market study on the quality characteristics of eggs from different housing systems. *Fd. Chem.,* **106**: 1031–1038. https://doi.org/10.1016/j.foodchem.2007.07.019

Hubbard, 2011. *Breeder nutrition guide*. Hubbard LLC, Walpole, MA.

Hulzebosch, J., 2006. Wide range of housing options for layers. *World's Poult.,* **22**: 20–22.

Huneau-Salaün, A., Guinebretière, M., Taktak, A., Huonnic, D. and Michel, V., 2011. Furnished cages for laying hens: study of the effects of group size and litter provision on laying location, zootechnical performance and egg quality: Animal. *Int. J. Anim. Biosci.,* **5**: 911–917. https://doi.org/10.1017/S1751731110002582

Khabisi, M.M., Salahi, A. and Mousavi, S.N., 2012. The influence of egg shell crack types on hatchability and chick quality. *Turk. J. Vet. Anim. Sci.,* **36**: 289–295. https://doi.org/10.3906/vet-1103-20

Khan, R.U., Rahman, Z.U., Javed, I. and Muhammad, F., 2013. Supplementation of dietary vitamins, protein and probiotics on semen traits and immunohistochemical study of pituitary hormones in zinc-induced molted broiler breeders. *Acta Histochem.,* **115**: 698– 704. https://doi.org/10.1016/j.acthis.2013.02.006

Khan, I.A. and Khan, S., 2018. Production performance of broiler breeders under cage versus floor housing systems. *Int. J. Biol.,* **13**: 449-462. https://doi.org/10.12692/ijb/13.1.449-462

Li, W., Li, H., Zhang, L., Hu, M., Li, F., Deng, J., An, M., Wu, S., Ma, R., Lu, J. and Zhou, Y., 2017. Long non-coding RNA LINC00672 contributes to p53 protein-mediated gene suppression and promotes endometrial cancer chemosensitivity. *J. biol. Chem.,* **292**: 5801–5813. https://doi.org/10.1074/jbc.M116.758508

Mahmoud, K.Z., Beck, M.M., Scheideler, S.E., Forman, M.F. anderson, K.P. and Kachman, S.D., 1996. Acute high environmental temperature and calcium-estrogen relationship in the hen. *Poult. Sci.,* **75**: 1555–1562. https://doi.org/10.3382/ps.0751555

McDaniel, C.D., Bramwell, R.K., Wilson, J.L. and Howarth, J.B., 1996. Fertility of male and female broiler breeders following exposure to elevated ambient temperatures. *Poult. Sci.,* **75**: 755-762. https://doi.org/10.3382/ps.0750755

Onagbesan, O.M., Metayer, S., Tona, K., Williams, J., Decuypere, E. and Bruggeman, V., 2006. Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. *Poult. Sci.,* **85**: 1245–1258. https://doi.org/10.1093/ps/85.7.1245

Pampori, Z.A. and Igbal, S., 2007. Haematology, serum chemistry and electrocardiographic evaluation in native chicken of Kashmir. *Int. J. Poult. Sci.,* **6**:

578-582. https://doi.org/10.3923/ijps.2007.578.582

Penfold, L.M., Wildt, D.E., Herzog, T.L., Lynch, W., Ware, L., Derrickson, S.E. and Monfort, S.L., 2000. Seasonal patterns of LH, testosterone and semen quality in the Northern Pintail duck. *Preprod. Fertil. Dev.,* **12**: 229-235. https://doi.org/10.1071/RD00093

Philippe, F.X., Mahmoudi, Y., Cinq-Mars, D., Lefrançois, M., Moula, N.,Palacios, J., Pelletier, F. and Godbout, S., 2020. Comparison of egg production, quality and composition in three production systems for laying hens. *Livest. Sci.,* **232**: 103917. https://doi.org/10.1016/j.livsci.2020.103917

Pirsaraei, Z.A., Shahneh, A.Z., Zaghari, M., Zamiri, M.J. and Mianji. G.R., 2008. Effect of testosterone and growth hormone injection before puberty on follicles size, rate of egg production and egg characteristics of the mazandaran native breeder hens. *Afr. J. Biotechnol.,* **7**: 3149–3154.

Pistekova, V., Hovorka, M., Vecerek, V., Strakova, E. and Suchy, P., 2006. The quality comparison of eggs laid by laying hens kept in battery cages and in a deep litter system. *Czech J. Anim. Sci.,* **5**: 318–325. https://doi.org/10.17221/3945-CJAS

Prabakaran, R., 2003. *Good practices in planning and management of integrated commercial poultry production in South Asia*. Chap. 3, FAO, Rome, Italy, pp. 9–24.

Roll, V.F.B., Briz, R.C. and Levrino, G.A.M., 2009. Floor versus cage rearing: Effects on production, egg quality and physical condition of laying hens housed in furnished cages. *Cienc. Rural,* **39**: 1527-1532. https://doi.org/10.1590/S0103-84782009000500034

Rossi, M., 2007. Influence of the laying hen housing systems on table egg characteristics. Proceedings of the XVIII European symposium on the quality of poultry meat and XII European symposium on the quality of eggs and egg products. *Prague,* **5**: 49–51.

SAS, 1996. *Statistical analysis system*. SAS System for Windows Version 6.12. SAS Institute Inc, Cary, NC 27513, USA.

Sayyazadeh, H. and Shahsavarani, H., 2005. *Effects of Artificial Insemination on performance of broiler breeders*. Department of Animal Science, Faculty of Agriculture, Mazandaran University, Sari, Iran. 4th European Poultry Genetics Symposium, Croatia

Shields, S. and Duncan, I.J.H., 2009. *A comparison of the welfare of hens in battery cages and alternative systems: Impacts on farm animals,* pp. 18. https://www.wellbeingintlstudiesrepository.org/hsus_reps_impacts_on_animals

Shields, S. and Greger, M., 2013. Animal welfare and food safety aspects of confining broiler chickens to cages. *Animals,* **3**: 386–400. https://doi.org/10.3390/ani3020386

Stanley, V.G., Nelson, D., Daley and M.B., 2013. Evaluation of two laying systems (Floor vs. Cage) on egg production, quality, and safety. *Iran. J. appl. Anim. Sci*., **4**: 597-601.

Starčević, M., Mahmutović, H., Glamočlija, N., Bašić, M. andjelković, R., Mitrović, R., Marković, R., Janjić, J., Bošković, M. and Baltić, M.Z., 2021. Growth performance, carcass characteristics, and selected meat quality traits of two strains of Pekin duck reared in intensive vs semi-intensive housing systems. *Int. J. Anim. Biosci.*, ***15***: 100087. https://doi.org/10.1016/j.animal.2020.100087

Tactacan, G.B., Guenter, W., Lewis, N.J., Rodriguez-Lecompte, J.C. and House, J.D., 2009. Performance and welfare of laying hens in conventional and enriched cages. *Poult. Sci.*, **88**: 698–707. https://doi.org/10.3382/ps.2008-00369

Tumova, E. and Ebeid, T., 2005. Effect of time of oviposition on egg quality characteristics in cages and in a litter housing system. *Czech J. Anim. Sci.,* **50**: 129–134. https://doi.org/10.17221/4006-CJAS

Voslarova, E., Hanzalek, Z., Vecerek, V., Strakova, E. and Suchý, P., 2006. Comparison between laying hen performance in the cage system and the deep litter system on a diet free from animal protein. *Acta Vet. Brno,* **75**: 219–225. https://doi.org/10.2754/avb200675020219

Walker, A.W. and Hughes, B.O., 1998. Egg shell colour is affected by laying cage design. *Br. Poult. Sci.,* **39**: 696-699. https://doi.org/10.1080/00071669888593

Massey, W.J., 2002. *Comparison of broiler breeder production and fertility in a colony cage system with two different floors versus a slat-floor system*. Graduate thesis, Dissertations, and Problem Reports. pp. 703.

World Health Organization, 2010. *WHO Laboratory Manual for the Examination and Processing of Human Semen* (5th ed), WHO Press, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/44261

Yakabu, A., Salako, A.E. and Ige, A.O., 2007. Effect of genotype and housing system on the laying performance of chickens in different seasons in semi-humid tropics. *Int. J. Poult. Sci.,* **6**: 434–439. https://doi.org/10.3923/ijps.2007.434.439

Yaniz, J.L., Marco-Aguado, M.A., Mateos, J.A. and Santolaria, P., 2010. Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm

quality during storage at 15°C. *Anim. Reprod. Sci.*, **122**: 142–149. https://doi.org/10.1016/j.anireprosci.2010.08.006

Yilmaz Dikmen, B., İpek, A., Şahan, U., Petek, M. and Sözcü, A., 2016. Egg production and welfare of laying hens kept in different housing systems (conventional, enriched cage, and free range). *Poult. Sci.,* **95**: 1564-1572. https://doi.org/10.3382/ps/pew082