

UNIVERSITI PUTRA MALAYSIA

PHYSIOLOGICAL AND BEHAVIOURAL RESPONSES TO DIFFERENT REARING SYSTEM IN BROILER CHICKENS

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PHYSIOLOGICAL AND BEHAVIOURAL RESPONSES TO DIFFERENT

REARING SYSTEM IN BROILER CHICKENS

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfilment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science)

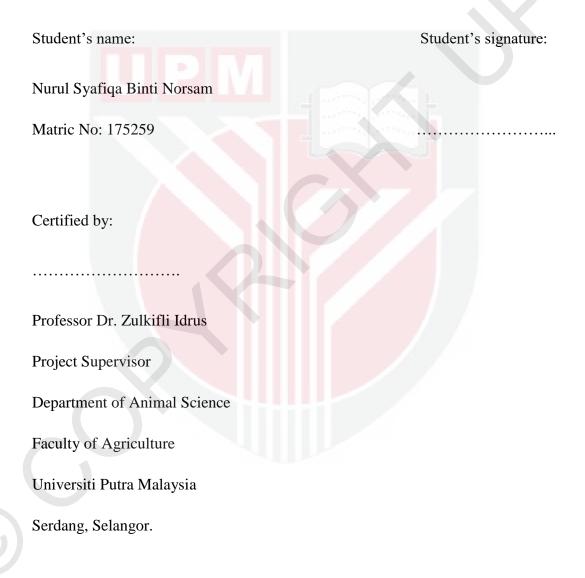
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CERTIFICATION

This project report entitled **Physiological And Behavioural Responses To Different Rearing System In Broiler Chickens** is prepared by **Nurul Syafiqa Binti Norsam** and submitted to the Faculty of Agriculture in fulfilment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science).



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TABLE OF CONTENT

CERTIFICATION	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENT	
LIST OF TABLES	v
LIST OF ABBREVIATIONS	vi
ABSTRACT	vii
ABSTRAK	ix
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	
2.1 Poultry Welfare	5
2.2 Stress, fear and immunological responses	7
2.3 Behavioural Responses	8
CHAPTER 3: MATERIALS AND METHODS	
3.1 Birds, housing and husbandry	10
3.2 Cage rearing system	10
3.3 Floor pen rearing system	11
3.4 Behaviour	11
3.5 Fearfulness	11
3.6 Blood parameters	12
3.7 Statistical analysis	14

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CHAPTER 4: RESULTS

4.1 Physiological Stress Measurements	15
4.2 Fearfulness	15
4.3 Immunoresponse	16
4.4 Behavioural Responses	16

CHAPTER 5: DISCUSSION

CHAPTER 6: CONCLUSION REFERENCE 18

21

22

LIST OF TABLES

 Table 1
 Welfare principles and criteria as defined by Welfare

 Quality

Table 2Table 2 Mean (±SEM) serum levels of corticosterone
(CORT), ovotransferrin (OVT) and ceruloplasmin
(CPN) in broiler chickens raised in cages and on floor
pens on day 42

15

15

6

- Table 3
 Table 3
 Mean (±SEM) number of inductions to induce tonic immobility and tonic immobility durations in broiler chicken raised in cages and on floor pens
- Table 4
 Mean (±SEM) percentages of feeding,

 drinking, walking, standing, resting, preening and
 object peaking in broiler chickens raised in cages and
 18

 on floor pens on day 13, 27 and 41.
 18

LIST OF ABBREVIATIONS

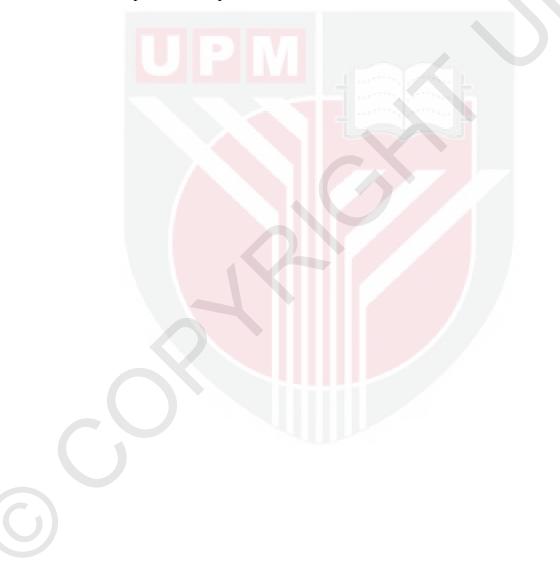
CORT	Corticosterone
APP	Acute Phase Protein
OVT	Ovotransferrin
CPN	Ceruloplasmin
TI	Tonic immobility
CRS	Cage rearing system
FRS	Floor rearing system
%	Percentage
С	Celcius
cm	Centimeter
m	Meter
S	second

ABSTRACT

Floor system is commonly practiced in the Malaysian broiler industry. In a hot and humid tropical environment, broilers tend to drink more and thus water intake increases. Higher water consumption may result in wet feces and lead to litter problem. Consequently irritation to mucous membrane, stress, increase susceptibility to diseases, contact dermatitis and leg weakness in broilers. The objective of this study was to determine the welfare of broiler chickens raised on litter floor pens and under the hot and humid tropical environment. Physiological, battery cages imunological and behavioural measurements were used to assess the welfare of broilers. A total of 200 one-day-old Cobb 500 male broiler chicks were equally assigned to either battery cages with wire floors or floor pens with wood shavings as litter material. There were 10 replicates of 10 birds for each rearing system. The cages and pens were in a naturally-ventilated house and the stocking density allowed for both rearing systems was 0.1 m²/bird. Birds were fed with commercial diet and vaccinated against Newcastle disease on day 7 and 21 via intraocular route. Broilers raised on floor pens had significantly higher corticosterone (CORT), ovotransferrin (OVT) and ceruloplasmin (CPN) than their cage rearing system (CRS) counterparts on day 42. Antibody titre against ND vaccinations on day 42 was not significantly affected by rearing system. Both CRS and FRS broilers showed similar durations of tonic immobility (TI) on day 42. However, the number of inductions to elicit TI was significantly higher in CRS birds when compared to those of FRS. Mean percentages of CRS chicks resting on day 13 was significantly higher than FRS. On day 13, the FRS birds showed significantly more walking activity than the CRS group. Other behaviors were not significantly affected by rearing system on day 13. On day 27, the

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CRS birds spent more time feeding and drinking than their FRS counterparts. Walking and resting activities were significantly reduced in CRS birds when compared to FRS on day 27. On day 41, only resting activity was significantly affected by rearing system. The FRS birds rested more than those of CRS. Based on physiological stress and fear reactions, this study suggested that cages, while may restrict the expression of a more diverse behavioural repertoire in broilers, were better than floor pens with respect to welfare.



ABSTRAK

Sistem lantai merupakan sistem yang biasa diamalkan di dalam industri ternakan ayam di Malaysia. Dalam persekitaran tropika panas dan lembap, ayam daging cenderung untuk minum lebih banyak air sekali gus meningkatkan pengambilan air. Pengambilan air yang lebih tinggi boleh mengakibatkan najis yang basah dan membawa kepada masalah sarap. Akibatnya kerengsaan kepada membran mukus, tekanan, meningkatkan kerentanan kepada penyakit, contact dermatitis dan kelemahan kaki dalam ayam daging. Kajian ini bertujuan untuk menentukan kebajikan ayam daging yang diternak di sarap lantai reban dan sangkar bateri di bawah persekitaran tropika yang panas dan lembap. Fisiologi, imunological dan pengukuran tingkah laku telah digunakan untuk menilai kebajikan ayam daging. Sejumlah 200 satu hari-Cobb 500 ayam jantan anak ayam diletakkan secara rawak sama ada sangkar bateri dengan jaring lantai atau lantai reban dengan kayu atau sebagai bahan sarap. Terdapat 10 replicates 10 burung bagi setiap sistem ternakan. Sangkar dan reban yang berada di rumah pengudaraan secara semula jadi dan kepadatan stok yang dibenarkan untuk kedua-dua sistem ternakan adalah 0.1 m2/burung. Burung diberi makan dengan diet yang komersial dan pelalian terhadap penyakit Newcastle pada hari 7 dan 21 melalui laluan intraocular. Ayam daging di lantai reban mempunyai jauh lebih tinggi CORT, OVT dan CPN daripada rakan-rakan CRS mereka pada hari 42. Titre antibodi terhadap ND vaksin pada hari 42 ini tidak ketara dipengaruhi oleh sistem ternakan. Ayam daging CRS dan FRS menunjukkan jangkamasa sama TI pada hari 42. Walau bagaimanapun, bilangan induksi menghimpunkan TI adalah jauh lebih tinggi dalam CRS burung jika dibandingkan dengan orang-orang yang FRS. Min peratusan anak ayam CRS berehat pada hari 13 adalah lebih tinggi dari FRS. Pada hari 13, burung

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FRS menunjukkan aktiviti berjalan kaki lebih daripada Kumpulan CRS. Tingkah laku yang lain tidak terjejas dengan ketara oleh sistem ternakan pada hari 13. Pada hari 27, burung CRS menghabiskan lebih banyak masa makan dan minum daripada ayam di FRS Berjalan dan berehat aktiviti telah ketaranya dalam CRS burung jika dibandingkan dengan FRS pada hari 27. Pada hari 41, hanya berehat aktiviti ini secara ketara dipengaruhi oleh penternakan sistem. Burung FRS berehat lebih daripada CRS. Berdasarkan reaksi ketakutan dan tekanan fisiologi, kajian ini mencadangkan bahawa sangkar, sementara mungkin mengehadkan tingkah laku yang pelbagai dalam ayam daging, adalah lebih baik daripada lantai reban dari segi kebajikan.

CHAPTER 1

INTRODUCTION

Rearing system is a crucial factor affecting the performance and welfare of poultry (Wang et al., 2015). One of the most common rearing system for broiler chickens in Malaysia is on floor pens with litter. However, the cost and availability of suitable litter materials for broiler production is a problem many areas. Litter scarcity and expense continue to increase, and substantial research is being directed towards finding alternatives to conventional litter grow-out of broilers. Cage system, which allow manure to pass through from birds, negate the need for litter and offer an obvious alternatives to conventional litter grow-out of boilers. Numerous workers have compared performance of broilers grown on litter with those raised in cages. Cage-reared broilers showed better growth performance when compared to those grown on litter when raised to heavy weights (Hypes et al., 1994). However, the welfare of broiler chickens in cages could be affected by movement restriction, poor bone strength leg disorders due to lack of exercise, and prevention of key behavioural patterns such as dustbathing and ground scratching (Shields and Greger, 2013). Broilers housed in cages were more susceptible to leg disorders (Haye and Simon, 1978; Rizk et al., 1980). There is a paucity of information on the effects of rearing system on physiological stress and fear reactions in broiler chickens. Work in laying hens showed negligible differences between cage and pen rearing systems in putative physiological measures such as adrenal, thyroid and pituitary weights, adrenal cholesterol, plasma glucose, cosrticosterone, or choloesteol, or differential leucocyte counts (Wolford and Ringer 1962; Bareham, 1972; Eskeland, 1976; Craig and Craig,

1985). The only documented work on physiological stress reaction to pen and cage rearing systems in broiler chickens was by Fouad *et al.* (2008). The authors found that caged birds had higher heterophil to lymphocyte ratios than those grown on floor pens. On a cautionary note, however, caged birds had smaller floor space when compared to those raised on floor pens (500 cm² versus 600 cm² per bird). Using novel object test, Fouad *et al.* (2008) reported that caged broilers were more fearful than their counterparts on floor pens.

Earlier work comparing broilers raised on litter and in cages were conducted under temperate conditions. In a hot tropical environment, chickens tend to drink more to remove the heat in the body (Boushy and Marle, 1978). Thus, they eliminate more water though the droppings and this will result in wet litter problem. Wet litter may increase obnoxious odour coming from ammonia and bacterial action in the droppings. Wet litter may compromise the welfare of chickens through irritation to mucous membrane, distress susceptibility to respiratory diseases, contact dermatitis and leg weakness (Ingrid *et al.*, 2014). Broiler chickens raised in cage rearing system may also provide a greater hygienic condition compared to litter rearing system (Willis *et al.*, 2002). A major advantage of cage rearing system is the separation of birds from their excreta and thus decreasing the disease and parasitic infection (Appleby *et al.*, 2004).

A central issue in animal welfare research is in how the welfare state of animals can be assessed objectively and scientifically. In the present study, the welfare of broilers raised on floor pens and in cages will be determined by physiological, behavioural and immunological measurements (Hughes and Curtis,

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1997; Mench and Mason, 1997; Terlouw et al., 1997). Circulating levels of corticosterone, which reflect the hypothalamic-pituitary-adrenal axis activity is considered the most frequently monitored physiological response to stress in poultry (Zulkifli and Siegel, 1995). Acute-phase proteins (APP) are a class of protein which in response to inflammation either the plasma concentrations increase (positive acutephase protein) or decrease (negative acute-phase protein). The changes in acute-phase protein concentration are due to changes in their production of hepatocytes. The positive APP includes ceruloplasmin (CPN) and ovotransferrin (OVT). Ceruloplasmin is a copper-containing ferroxidase which protects tissues from iron-mediated free radical injury through oxidation of the toxic ferrous iron to nontoxic ferric form (Patel et al., 2002). Ovotransferrin is an iron binding protein and provide antimicrobial properties by sequestering iron and also to modulate heterophil and macrophage function in chickens (Murata et. al., 2004). Recent work showed that serum levels of APP were modified in chickens subjected to adverse non-inflammatory stimuli such as high temperature (Najafi et al., 2015; Olubodun et al., 2015), overcrowding (Shakeri et al., 2014; Najafi et al., 2015), and feed withdrawal (Najafi et al., 2016). Murata *et al.*, (2014) suggested that APP may play a profound role in the restoration of homeostasis in animals with respect to non-inflammatory, psychophysical stressors. encountering difficult conditions often show Animals some degree of immunosuppression. Stress influences both cell mediated and antibody dependent immune functions (Kidd, 2004).

Behavior is the internally coordinated and externally visible response of whole living organism towards the internal or external stimuli. Behaviour is considered one of the most easily observed measures of welfare. Animals respond to environmental difficulties by altering their behaviour . Abnormal behavior is behaviour that differs in pattern, frequency or context from which is shown by most members of the species in conditions that allow a full range of behavior (Fraser and Broom, 1997). Fear is an undesirable state of suffering and a powerful stressor which can seriously harm an animal's welfare (Jones, 2002). Tonic immobility, a temporary state of motor inhibition, loss of the righting response and reduced responsiveness to the external stimulation has been widely used to determine underlying fearfulness in poultry (Zulkifli *et al.*, 2009; Al-Aqil and Zulkifli *et al.*, 2009; Al-Aqil *et al.*, 2013).

The objective of this study was to determine the welfare of broiler chickens raised on litter floor pens and battery cages under the hot and humid tropical environment. Physiological, imunological and behavioural measurements were used to assess the welfare of broilers.

REFERENCE

- Al-Aqil, A., Zulkifli, I., Bejo, M. H., Sazili, A. Q., Rajion, M. A., & Somchit, M. N. (2013). Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. *Poultry science*, 92(1), 33-40.
- Al-Murani, W.K., A. Kassab, H.Z. Alsam and A.M.K. Althari, 1997:
 Heterophil/Lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. Br. Poult. Sci., 38: 159-163.
- Appleby, M.C. Mench, J.A. and Hughes, B.O. 2004. Poultry Behaviour and Welfare, Cambridge: CABI Publishing.
- Bareham, J.R., 1972. Effects of cages and semi-intensive deep litter pens on the behaviour adrenal response and production in two strains of laying hens. Br. Vet. J. 128, 153–163.
- Barnett, J. L., & Hemsworth, P. H. (2009). Welfare monitoring schemes: using research to safeguard welfare of animals on the farm. Journal of Applied Animal Welfare Science, 12(2), 114-131.
- Benoff, F. H., & Siegel, P. B. (1976). Genetic analysis of tonic immobility in youngJapanese quail (Coturnix cotunix japonica). *Animal learning & behavior*, 4(2), 160-162.
- Bessei, W. (2006). Welfare of broilers: a review. World's Poultry Science Journal, 62(03), 455-466.

- Blokhuis, H. J. (1983). The relevance of sleep in poultry. World's Poultry Science Journal, 39(01), 33-37.
- Blokhuis, H. J., & Van der Haar, J. W. (1990). The effect of the stocking density on the behaviour of broilers. Archiv für Geflügelkunde, 54(2), 74-77.
- Blokhuis, H.J., 1984. Rest in poultry. Appl. Anim. Behav. Sci. 12, 289-303.
- Boushy, A.R.E. and van Marle, A.L. (1978) 'The Effect of Climate on Poultry Physiology in Tropics and their Improvement', *World's Poultry Science Journal*, 34(3), pp. 155–171
- Broom, D. M., & Johnson, K. G. (1993). Stress and animal welfare. Springer Science & Business Media.
- Campo, J. L., & Carnicer, C. (1993). Realized heritability of tonic immobility in White Leghorn hens: a replicated single generation test. *Poultry science*, 72(12), 2193-2199.
- Coleman, G. J., Hemsworth, P. H., & Hay, M. (1998). Predicting stockperson behaviour towards pigs from attitudinal and job-related variables and empathy. Applied Animal Behaviour Science, 58(1), 63-75.
- Craig, J. V., and J. A. Craig, 1985. Corticosteroid levels in White Leghorn hens as affected by handling, laying-house environment, and genetic stock. Poultry Sci. 64:809–816.

- Cunningham, D. L., & Mauldin, J. M. (1996). Cage housing, beak trimming, and induced molting of layers: A review of welfare and production issues. The Journal of Applied Poultry Research, 5(1), 63-69.
- Dawkins, M. S. (1989). Time budgets in red junglefowl as a baseline for the assessment of welfare in domestic fowl. *Applied Animal Behaviour Science*, 24(1), 77-80.
- de Jong, I. C., Gunnink, H., & Van Harn, J. (2014). Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance, and carcass yield in broiler chickens. The Journal of Applied Poultry Research, 23(1), 51-58.
- De Jong, I., Berg, C., Butterworth, A., & Estevéz, I. (2012). Scientific report updating the EFSA opinions on the welfare of broilers and broiler breeders. *Support. Publ.* 2012EN-295, 1-116.
- Eskeland, B. (1976). Methods of observation and measurement of different parameters as an assessment of bird welfare. In *5 th European Poultry Conference, Malta* (Vol. 2, pp. 988-998).
- Fouad, M.A., Razek, A.H.A., Badawy, S.M., 2008. Broiler welfare and economics under two management alternatives on commercial scale. *Int. J. Poult. Sci.* 7, 1167-1173.
- Fraser, A. F., & Broom, D. M. (1997). *Farm animal behaviour and welfare* (No. Ed.3). CAB international.

- Fraser, D., & Duncan, I. J. (1998). 'Pleasures', 'pains' and animal welfare: toward a natural history of affect.
- Gentle, M. J., Jones, R. B., & Maguire, S. (1985). Telencephalic removal and tonic immobility in the domestic hen (Gallus domesticus). *Behavioural* processes, 10(3), 265-271.
- Haye, U. Simon, P.C.M. Twisted legs in broilers. Br. Poul. Sci. 1978, 19, 549-557.
- Hughes, B. O., & Curtis, P. E. (1997). Health and disease. In M. C. Appleby, & B. O.Hughes (Eds.), Animal welfare (pp. 109e125). Wallingford, UK: CABInternational,.
- Hypes, W.A. Carpenter, G.H. Peterson, R.A. Jones, W.T. Productive performance of conventional floor-reared broilers vs. high density cage-brooded broilers. J. Appl. Poul. Res. 1994, 3, 238-243.
- Johnson, R. W., Curtis, S. E., & Shanks, R. D. (1991). Effects on chick performance of ammonia and heat stressors in various combination sequences. Poultry science, 70(5), 1132-1137.
- Jones, B. R. (2002). Role of comparative psychology in the development of effective environmental enrichment strategies to improve poultry welfare. *International journal of comparative psychology*, 15(2).
- Jones, R. B. (1984). Experimental novelty and tonic immobility in chickens (Gallus domesticus). *Behavioural processes*, 9(2), 255-260.

- Jones, R. B. (1986). The tonic immobility reaction of the domestic fowl: a review. *World's poultry science journal*, 42(01), 82-96.
- Jones, R. B. (1996). Fear and adaptability in poultry: insights, implications and imperatives. *World's Poultry Science Journal*, 52(02), 131-174.
- Kidd, M. T. (2004). Nutritional modulation of immune function in broilers. *Poultry Science*, 83(4), 650-657.
- Konaka, S., Ohashi, H., Okada, T. & Takewaki, T. (1979). Appearance of noradrenaline and adrenaline and the developmental changes in their concentrations in the gut of the chick. British Journal of Pharmacology, 65: 257– 260
- Kuenzel, W. J., Douglass, L. W., & Davison, B. A. (1987). Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, Gallus domesticus. *Peptides*, 8(5), 823-828.
- Lam, K. M., Kabbur, M. B., & Eiserich, J. P. (1996). Newcastle disease virus-induced functional impairments and biochemical changes in chicken heterophils. Veterinary immunology and immunopathology, 53(3), 313-327.
- LEHNER, P. N. (1992). Sampling methods in behavior research. Poultry science, 71(4), 643-649.
- Mancini G, Carbonara AO, Heremans JF (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem 2:235–254

- Martinez- Subiela, S., Eckersall, P. D., Campbell, F. M., Parra, M. D., Fuentes, P., & Ceron, J. J. (2007). A time- resolved immunofluorometric assay for porcine
 C- reactive protein quantification in whole blood. *Luminescence*, 22(3), 171-176.
- Mashaly, M. M., Hendricks, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O., & Patterson, P. H. (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science*, 83(6), 889-894.
- Maxwell, M. H. (1993). Avian blood leucocyte responses to stress. World's Poultry Science Journal, 49(01), 34-43.
- Mench, J. A., Van Tienhoven, A., Marsh, J. A., McCormick, C. C., Cunningham, D. L., & Baker, R. C. (1986). Effects of cage and floor pen management on behavior, production, and physiological stress responses of laying hens. *Poultry Science*, 65(6), 1058-1069.
- Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J. 168:28–40.
- Najafi, P., Zulkifli, I., Soleimani, A. F., & Goh, Y. M. (2016). Acute phase proteins response to feed deprivation in broiler chickens. Poultry science, pew001.
- Najafi, P., Zulkifli, I., Soleimani, A. F., & Kashiani, P. (2015). The effect of different degrees of feed restriction on heat shock protein 70, acute phase proteins, and other blood parameters in female broiler breeders. Poultry science, pev246.
- Olubodun, J. O., Zulkifli, I., Farjam, A. S., Hair-Bejo, M., & Kasim, A. (2015). Glutamine and glutamic acid supplementation enhances performance of broiler

chickens under the hot and humid tropical condition. *Italian Journal of Animal Science*, *14*(1), 3263.

Patel, B. N., Dunn, R. J., Jeong, S. Y., Zhu, Q., Julien, J. P., & David, S. (2002). Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. The Journal of Neuroscience, 22(15), 6578-6586.

Poultry Behaviour. Retrieved 9 December 2016 from
http://www.poultryhub.org/production/husbandry-management/poultry-behaviour/

- Poultry Welfare in Developing Coutries. Retrived 9 December 2016 from http://www.fao.org/docrep/019/i3531e/i3531e09.pdf
- Reynolds, D. L., & Maraqa, A. D. (2000). Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. Avian diseases, 138-144.
- Rizk, S.W. Stake, P.E. Simmons, R.W. III. Curled toes and perosis-like leg abnormalities in cage reared broilers. *Poul. Sci.* 1980, 59, 308-315.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. Endocrine reviews, 21(1), 55-89.
- Shakeri, M., Zulkifli, I., Soleimani, A. F., o'Reilly, E. L., Eckersall, P. D., Anna, A.A., ... & Abdullah, F. F. J. (2014). Response to dietary supplementation of l-glutamine and l-glutamate in broiler chickens reared at different stocking

densities under hot, humid tropical conditions. Poultry science, 93(11), 2700-2708.

- Shields S. and Greger M., 2013. Animal Welfare and Food Safety Aspects of Confining Broiler Chicken to Cages. In: Animal, Washington, USA, pp.386-400.
- Terlouw E M C, Schouten W G P and Ladewig J 1997 Physiology. In: Appleby M C and Hughes B O (eds) Animal Welfare pp 143-158. CAB International: Wallingford, UK
- Wang, Y. M., Meng, Q. P., Guo, Y. M., Wang, Y. Z., Wang, Z., Yao, Z. L., & Shan,
 T. Z. (2010). Effect of atmospheric ammonia on growth performance and immunological response of broiler chickens. *Journal of Animal and Veterinary Advances*, 9(22), 2802-2806.
- Wang, Y., Ru, Y. J., Liu, G. H., Chang, W. H., Zhang, S., Yan, H. J., ... & Cai, H. Y. (2015). Effects of different rearing systems on growth performance, nutrients digestibility, digestive organ weight, carcass traits, and energy utilization in male broiler chickens. *Livestock Science*, 176, 135-140.
- Weaver, W. D., & Meijerhof, R. (1991). The effect of different levels of relative humidity and air movement on litter conditions, ammonia levels, growth, and carcass quality for broiler chickens. *Poultry Science*, 70(4), 746-755.
- Weeks, C. A., Danbury, T. D., Davies, H. C., Hunt, P., & Kestin, S. C. (2000). The behaviour of broiler chickens and its modification by lameness. *Applied animal behaviour science*, 67(1), 111-125.

- Weeks, C. A., Nicol, C. J., Sherwin, C. M., & Kestin, S. C. (1994). Comparison of the behaviour of broiler chickens in indoor and free-range environments. Animal Welfare, 3(3), 179-192.
- Wegner, R.M., 1992. Analysis of the Research into Alternative Systems. In: Carter, H. and V. Carter (Eds.). The laying Hen, (Brussels, European conference group on the protection of farm animals), pp: 45-55.
- Willis, W. L., Murray, C., & Talbott, C. (2002). Campylobacter isolation trends of cage versus floor broiler chickens: a one-year study. Poultry science, 81(5), 629-631.
- Willis, W.L. Murray, C. Talbott, C. Campylobacter isolation trends of cage versus floor broiler chickens: A one-year study. *Poul. Sci.* 2008, 87, 405-420.
- Wolford, J. H., & Ringer, R. K. (1962). Adrenal weight, adrenal ascorbic acid, adrenal cholesterol and differential leucocyte counts as physiological indicators of "stressor" agents in laying hens. *Poultry science*, 41(5), 1521-1529.
- Zulkifli, I., & Siegel, P. B. (1995). Is there a positive side to stress?. World's Poultry Science Journal, 51(01), 63-76.
- Zulkifli, I., Al-Aqil, A., Omar, A. R., Sazili, A. Q., & Rajion, M. A. (2009). Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. *Poultry science*, 88(3), 471-476.

- Zulkifli, I., Najafi, P., Nurfarahin, A. J., Soleimani, A. F., Kumari, S., Aryani, A. A., ...
 & Eckersall, P. D. (2014). Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone. *Poultry science*, *93*(12), 3112-3118.
- Zulkifli, I., Norma, M. C., Chong, C. H., & Loh, T. C. (2000). Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. *Poultry Science*, *79*(3), 402-406.

