***EFFECT OF FEEDING DIFFERENT LEVELS OF DL-METHIONINE AND L-METHIONINE ON HEAT SHOCK PROTEIN 70 EXPRESSION AND BLOOD PARAMETERS IN BROILER CHICKENS UNDER HEAT STRESS CONDITION***

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**FP 2016 83**

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**2015/2016**

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**A Project report Submitted to Faculty of Agriculture,**

**Universiti Putra Malaysia,**

**In Partial Fulfillment of the Requirement of SHW 4999**

**(Final Year Project)**

**For the Award of the Degree of**

**Bachelor of Agriculture (Animal Science)**

**Department of Animal Science**

**Faculty of Agriculture**

**Universiti Putra Malaysia**

**Serdang, Selangor**

## CERTIFICATION

The project report attached here entitled:

**Effect of Feeding Different Levels of DL-Methionine and L-Methionine on Heat Shock Protein 70 Expression and Blood Parameters in Broiler Chickens under Heat Stress Condition** and submitted by **Meenambigay A/P Nagaraju**

In partial fulfillment or the requirement of SHW 4999 (final year project) for the award of the degree of **Bachelor of Agriculture (Animal Science)** is hereby accepted.

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Serdang, Selangor. Date: ________________

# ACKNOWLEDGEMENT

First and foremost, I would like to express my great gratitude and deep appreciation to my supervisor, Professor Dr. Zulkilfli bin Idrus, for his scholastic guidance, invaluable suggestions, kind cooperation and enthusiasm in course of my study. Whilst carrying out duties or responsibilities, his precious comments and criticisms, thorough corrections and enthusiastic cooperation, have enabled me to complete my research work successfully.

I am happy to stretch out my cordial thanks to Dr. M. A. Hossain (Rony), Post-Doctoral Fellow, Institute Tropical Agriculture, University Putra Malaysia, for his kind cooperation and help from different aspects during my study period. Furthermore, I could not but acknowledge the help and assistance of other personnel of this University (UPM) namely Ainil Fatin Bt Akmal and Norasyikin Bt Mochamat, postgrad students, all the staffs of Animal Research Centre and Laboratory of Animal Production, for continuous assistance and providing me a good facilities throughout my research study.

I am pleased to give special thanks to my entire friends, who had helped me directly or indirectly, by sharing knowledge and ideas time to time in order to make this research work fruitful.

After all, my heartfelt appreciation, gratitude and thank from the core of my heart go to my beloved parents, who gave me endless moral and financial supports all the time of my study period in this university.

Thank you.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **%** | percentage |
| **°C** | degree celsius |
| **µl** | microlittre |
| **AA** | amino acid |
| **AGP** | α-1-acid glycoprotein |
| **ANOVA** | Analysis of Variance |
| **APP** | acute phase proteins |
| **APR** | acute phase response |
| **C** | degree celcius |
| **CORT** | corticosterone |
| **CP** | crude protein |
| **CRD** | Complete Randomized Design |
| **d** | day |
| **DCP** | dicalcium phosphate |
| **DL-Met** | DL-Methionine |
| **DMRT** | Duncan's Multiple Range Test |
| **kcal ME/kg** | kilocalorie Metabolizable Energy/kilogram |
| **Kg** | kilogram |
| **L-Met** | L-Methionine |
| **ME** | Metabolizable Energy |
| **mg/ml** | milligram per milliliter |

| | |
|---|---|
| **MJ** | mega joule |
| **ml** | milliliter |
| **ng/ml** | nanogram per milliltre |
| **nm** | Nanometer |
| **OVT** | Ovotransferin |
| **h** | hour |
| **rpm** | revolutions per minute |
| **SAS** | Statistical analytical system |
| **SDS** | sodium dodecyl sulfate |
| **SDS-page** | SDS polyacrylamide gel electrophoresis |
| **TBS** | Tris-Buffered Saline |
| **TMB** | tetramethylbenzidine |
| **Tris** | trisaminomethane hydrochloride |
| **UPM** | Universiti Putra Malaysia |

**ABSTRACT**

Methionine (Met) is a sulphur-containing essential amino acid (AA), and is always supplemented in poultry diet to enhance production. A study was conducted to compare the effects of various levels of DL-Methionine (DL-Met) and L-Methionine (L-Met) (feed grade) on physiological reactions to high ambient temperatures in broiler chickens. A total of 660 one-day-old male broiler chicks (Cobb x Cobb) were used in this study. Birds were randomly assigned in a group of five to 132 battery cages. All birds received a standard broiler starter diet from day 1 to 10 and standard grower diet from day 11 to 21. From day 22 to 42, equal number of birds (12 cages / diet) were fed isocaloric and isonitrogenous diets with various levels of DL-Met or L-Met supplementation; (i) 0% DL-Met or L-Met (as a negative control), (ii) 0.136% DL-Met, (iii) 0.136% L-Met. (iv) 0.153% DL-Met, (v) 0.153% L-Met, (vi) 0.170% DL-Met, (vii) 0.170% L-Met, (viii) 0.187% DL-Met, (ix) 0.187% L-Met, (x) 0.204% DL-Met, or (xi) 0.204% L-Met. For each dietary group, equal numbers of birds (6 cages per diet-temperature subgroup) were subjected to unheated (23$^{o}$C) or heated (32$^{o}$C for 6 hours/day) condition. On day 42, 2 birds per cage were randomly selected, killed and, blood [to determine serum levels of corticosterone (CORT) and acute phase proteins [ovotransferin (OVT) and α-1-acid glycoprotein (AGP)] and liver [to determine heat shock protein (HSP) 70 density] samples were collected. Irrespective of diet, heat treatment elevated CORT, OVT, and AGP. Diet had no significant effect on all those parameters. There were significant diets x temperature interactions for HSP 70 density. Birds that were not supplemented with either DL-Met or L-Met were not able to express HSP 70 following heat treatment. In

conclusion, other than HSP 70, there was little evidence that L-Met is more efficient than DL-Met, and higher level of methionine supplementation did not improve heat tolerance in chickens.

# ABSTRAK

Methionine merupakan amino asid (AA) yang mengandungi sulfur, dan ia sentiasa disuplimenkan dalam pemakanan ayam. Tujuan utama kajian ini dijalankan untuk mengkaji kesan pelbagai peringkat DL- Methionine (DL -Met) dan L- Methionine (L-Met) ( gred makanan haiwan ) pada tindak balas fisiologi kepada suhu ambien yang tinggi pada ayam pedaging. 660 sehari berusia anak ayam daging jantan ( Cobb x Cobb ) telah digunakan dalam kajian ini. Burung diagihkan secara rawak dalam satu kumpulan lima ekor ayam ke 132 sangkar bateri. Semua burung menerima diet starter standard dari hari 1 hingga 10 dan diet grower dari hari 11 hingga 21. Dari hari 22-42 , jumlah yang sama burung (12 sangkar / diet) diberi makan diet isocaloric dan isonitrogenous dengan pelbagai peringkat DL -Met atau suplemen L -Met ; (i) 0% DL-Met or  L-Met  (as a negative control), (ii) 0.136% DL-Met, (iii) 0.136% L-Met. (iv) 0.153% DL-Met, (v) 0.153% L-Met, (vi) 0.170% DL-Met, (vii) 0.170% L-Met, (viii) 0.187% DL-Met, (ix) 0.187% L-Met, (x) 0.204% DL-Met, or  (xi) 0.204% L-Met. Bagi setiap kumpulan diet, jumlah burung yang sama (6 sangkar setiap diet -suhu subkumpulan ) tertakluk kepada tak panas ( 23˚C ) atau panas ( 32˚C selama 6 jam / hari). Pada hari ke 42, 2 burung setiap sangkar telah dipilih secara rawak , dibunuh secara berperikemanusian dan, darah dikumpulkan [ untuk menentukan tahap serum corticosterone ( CORT ) dan protein akute phase [ ovotransferin ( OVT ) dan alpha-1- asid Glikoprotein (AGP )] dan hati [ untuk menentukan heat shock protein ( HSP) 70 densiti ]. Tanpa mengira diet, rawatan haba meningkat dalam CORT, OVT, dan AGP . Diet tidak mempunyai kesan yang penting ke atas semua parameter tersebut. erdapat ketara interaksi diet x suhu untuk HSP

70 densiti. Burung yang tidak ditambah dengan DL -Met atau L -Met tidak mampu untuk menghasilkan HSP 70 selepas rawatan haba. Kesimpulannya, selain daripada HSP 70, terdapat sedikit bukti bahawa L -Met adalah lebih cekap daripada DL -Met, dan tahap yang lebih tinggi daripada suplemen methionine tidak bertambah baik toleransi haba pada ayam.

# CHAPTER 1

# INTRODUCTION

Methionine (Met) is an essential amino acid for poultry and it is the first limiting AA in corn-soy diets (Dilger and Baker, 2007). Met has various biological functions that are important for the development and health status of animals (Bunchasak, 2009). Commercial poultry diets are commonly supplemented with dry DL-methionine (DLM; 99% pure) or as liquid DLM hydroxy analogue free acid (MHA-FA, containing 88% of active substance). Met can exist in either the L-or D-isomeric forms called enantiomers, which are mirror images of each other. Chemically, there is no difference between the two forms but enzymes prefer the L-form. Only the L-isomers of amino acids are incorporated into bio-active proteins, and L-Met delivers 100% the L-form of met. The D-isomer from DL-methionine, and both of the D- and L-isomers from methionine hydroxy analogue (MHA), must be converted into the L-form by a two-step enzymatic process that is never 100% efficient, resulting in metabolic losses. (Shen *et al.*, 2015). Feed grade L-methionine was recently introduced commercially (CJ Cheil Jedang Co., Seoul, Korea). It was claimed that L-Met is 10 % more efficient than DL-Met. (Shen *et al.* 2015) reported that L-Met supplementation served a better function on redox status, development of the gut of young chicks, and growth performance compared with DL-Met.

There is, however, a lack of information on the bio-efficacy of L-Met in broiler chickens under heat-stress condition. Methionine requirements may be affected by high temperatures. In the hot regions of the world, heat stress is a major concern in poultry production. Reductions in growth performance and survivability of broiler chickens with increases in climatic temperature have been well documented (Gous and Morris 2005). The effect of various stressors on the hypothalamic-pituitary-adrenal axis and the consequent elevation in circulating level of corticosterone in poultry is well established (Zulkifli and Siegel, 1995). Hence, changes in plasma or serum corticosterone concentration (CORT) have been commonly used to assess physiological reaction to heat challenge in poultry (Zulkifli *et al.,* 2011; Soleimani *et al.,* 2011). Acute phase proteins (APP) are a group of proteins that are primarily synthesised in the hepatocytes and released into the bloodstream by a variety of challenges such as bacterial infection, inflammation, tissue injury, endotoxin exposure, and neoplasia (Murata *et al.* 2004; O'Reily and Eckersall 2014). The functions of APP included protease inhibitors, enzymes, transport proteins, coagulation proteins, and modulators of the immune response. α1-acid glycoprotein is a sialoglycoprotein produced and then secreted typically by hepatocytes. The protein was associated with homeostasis maintenance through reduction of tissue damage related to inflammatory response in extrahepatic cells (Fournier *et al.* 2000). Although ovotranferrin is typically specified as a negative APP, there are some evidences that chicken serum ovotranferrin concentration increased in inflammation response (Tohjo *et al.* 1995; Xie *et al.* 2002). Ovotranferrin may be included in innate immune system by sequestration of ferric ions to prevent of parasites and also pathogens from using nutrients (Law, 2002). According to Murata *et al.* (2004), APPs play a profound role in the restoration of homeostasis in animals subjected to non-inflammatory, psychophysical stressors. Although there is considerable work on APP response to stressors in cattle (Arthington *et al.* 2003) and pigs (Pineiro *et al.* 2007) little information is available on the effect of environmental stressors on APP in poultry. Recent work (Shakeri *et* *al.* 2014) suggested that broiler chickens stocked at 0.067 $m^2$ / birds on deep litter showed elevated serum levels of α1-acid glycoprotein (AGP), and ovotransferrin (OVT), when compared to those housed at 0.100 $m^2$ / bird.

Living organisms respond to thermal and non-thermal stressors by synthesizing a group of highly conserved proteins known as heat shock proteins (HSP) (Soleimani *et al*. 2012b). It is well documented that HSP play a profound role in modifying physiological stress response and in acquisition of stress tolerance (Kregel, 2002). Work in chickens indicated that heat challenge (Soleimani *et al.*, 2011) may elicit HSP 70 expression.

**1.1 Objective**

The study was carried out to compare the effects of various levels of feed grade DL-Met and L-Met on CORT, OVT, AGP and liver HSP 70 density in heat-stressed broiler chickens.

**1.2 Research hypothesis**

Supplementation with feed grade L-Met would have better effects on physiological responses to heat challenge in broiler chickens compared with the use of feed grade DL-Met.

**1.3 Significance of study**

Results from this study will be important to determine the optimum level of feed grade L-Met and feed grade DL-Met in broiler chickens under heat stress condition. This is important to ensure economic feasibility of poultry feed production.

# BIBLIOGRAPHY


Allyn, S. (2014). Detection of Inducible HSP70 as a Measure of Heat Stress in Mammalian Tissue.

Al-Aqil A., and Zulkifli I. (2009). Changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poultry Science., 88:1358-1364.

Arthington J, Eicher S, Kunkle WE, Martin FG (2003) Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. J AnimSci 81:1120–1125

Baker, D.H. (1994). Utilization of precursors for L-amino acids. In: DíMello JPF, editor. Amino acids in farm animal nutrition. Wallingford, UK: CAB International;. p.37-62

Barbe, M. F., Tytell, M. D., Grower, D. J. & Welch, W. J. (1988). Hyperthermia protects against light damage in rat retina. Science, 241:1817–1820.

Bouyeh, M. (2012). Effect of Excess Lysine and Methionine on Immune system and Performance of Broilers. Annals of Biological Research, 3 (7):3218-3224.

Brosnan, J.T. and Brosnan, M.E. (2006). The sulfur-containing amino acids: an overview. The Journal of Nutrition, 136: 1636S–1640S.

Bunchasak, C, & Silpasorn T. (2005). Effects of adding methionine in low protein diet on production performance, reproductive organs and chemical liver composition of laying hens under tropical conditions. International Journal of Poultry Science, 4:301-308.

Bunchasak, C. (2009). Role of dietary methionine in poultry production. Journal of Poultry Science, 46:169-179.

Carroll, J. A., & Forsberg, N. E. (2007). Influence of stress and nutrition on cattle immunity. Veterinary Clinics of North America: Food Animal Practice, 23(1), 105-149.

Carsia, R. V., and S. Harvey. (2000). Adrenals. Pages 489-537 in Sturkie's avian physiology. G. Whittow ed. Academic Press, New York.

Champagne, D.L., Bagot, R.C., van Hasselt, F., Ramakers, G., Meaney, M.J., de Kloet, E.R., Joels, M. & Krugers, H. (2008). Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. The Journal of Neuroscience, 28, 60376045.

Cobb-Vantress, (2012). Cobb 500TM: broiler performance and nutrition supplement guide. Available from: http://www.ugachick.co/ manuals/ Cobb500ManNutriPg.pdf.

Cooke, R., J. Arthington, B. Austin, and J. Yelich. (2009). Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahmancrossbred heifers. J. Anim. Sci. 87:3403-3412.

Cray, C., Zaias, J., & Altman, N. H. (2009). Acute phase response in animals: a review. Comparative medicine, 59(6), 517.

Dilger, R. N., & Baker, D. H. (2007). DL-Methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in sulfur amino acid-deficient purified and practical-type diets fed to chicks. Poultry science, 86(11), 2367-2374.

Etches, R. J. , T. M. John, and A. M. Verrinder Gibbins. (1995). Behavioural, physiological, neuroendocrine and molecular responses to heat stress. Pages 31–65 in Poultry Production in Hot Climates. D. J. Daghir, ed. CAB International, Wallingford, UK.

Fournier, T., Medjoubi-N, N., & Porquet, D. (2000). Alpha-1-acid glycoprotein. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1482(1), 157-171.

Georgieva, T. M., Koinarski, V. N., Urumova, V. S., Marutsov, P. D., Christov, T. T., Nikolov, J., ... & Koinarski, Z. V. (2010). Effects of Escherichia coli infection and Eimeria tenella invasion on blood concentrations of some positive acute phase proteins (haptoglobin (PIT 54), fibrinogen and ceruloplasmin) in chickens. Revue de medecine veterinaire, 161(2), 84.

Geraert, P. A., S. Guillaumin and B. Leclercq. (1993). Are genetically lean broilers more resistant to hot climate? Br. Poult. Sci. 34(4):643-653.

Gething, M. J., and J. Sambrook. (1992). Protein folding in cell. Nature 355:33–35

Gonzalez-Esquerra, R., & Leeson, S. (2006). Physiological and metabolic responses of broilers to heat stress-implications for protein and amino acid nutrition. World's Poultry Science Journal, 62(02), 282-295.

Gous, R.M. and T.R. Morris,(2005). Nutritional interventions in alleviating the effects of high temperatures in broiler production. World's Poult. Sci. J., 61: 463-475

Knight, C. D., Wuelling, C. W., Atwell, C. A., & Dibner, J. J. (1994). Effect of intermittent periods of high environmental temperature on broiler performance responses to sources of methionine activity. Poultry Science, 73(5), 627-639.

Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J ApplPhysiol 92:2177–2186

Law JH (2002) Insects, oxygen, and iron. Biochemical and Biophysical Res Communications 292:1191–1195

Leeson, S and Summers, J.D. (2001). Nutrition of the chicken. 4th Edn; pp:112-125.

Lemme, A., Hoehler, D., Brennan, J. J., & Mannion, P. F. (2002). Relative effectiveness of methionine hydroxy analog compared to DL-methionine in broiler chickens. Poultry science, 81(6), 838-845.

Liew, P. K., Zulkifli, I., Hair-Bejo, M., Omar, A. R., & Israf, D. A. (2003). Effects of early age feed restriction and heat conditioning on heat shock protein 70 expression, resistance to infectious bursal disease, and growth in male broiler chickens subjected to heat stress. Poultry science, 82(12), 1879-1885.

Mahmoud, K. Z., Edens, F. W., Eisen, E. J., & Havenstein, G. B. (2004). Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (Gallus gallus domesticus) subjected to cyclic heat stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 137(1), 35-42.

Mancini, G. A., Carbonara, A. T., & Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2(3), 235-IN6.

McEwen, B. S., De Kloet, E. R., & Rostene, W. H. (1986). Adrenal steroid receptors and actions in the nervous system. Physiology Review, 66, 1121-1188.

Mora, F., Segovia, G. Alberto del Arcoa, Marta de Blasa, Pedro Garrido (2012). Stress, neurotransmitters, corticosterone and body–brain integration. Brain Research , 1476: 71-85.

Morimoto, R. I. (1993). Cells in stress: transcriptional activation of heat shock genes. Sci. 259:1409-1410.

Multhoff, G. (2007). Heat shock protein 70 (Hsp70): membrane location, export and immunological relevance. Methods, 43: 229-237

Murata H, Shimada N, Yoshioka M (2004) Current Research on Acute Phase Proteins in Veterinary Diagnosis: An Overview. Veterinary Journal, 168:28–40.

Najafi, P., Zulkifli, I., Jajuli, N. A., Farjam, A. S., Ramiah, S. K., Amir, A. A., ... & Eckersall, D. (2015). Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. International journal of biometeorology, 1-7.

Noll SL, Waibel PE, Cook DR, Witmer JA. (1984). Biopotency of methionine sources for young turkeys. Poultry Science; 63:2458-2470.

O'REILLY, E. L., & Eckersall, P. D. (2014). Acute phase proteins: a review of their function, behaviour and measurement in chickens. World's Poultry Science Journal, 70(01), 27-44.

Pineiro M, Piñeiro C, Carpintero R, Morales J, Campbell FM, Eckersall PD, Toussaint MJ, Lampreave F (2007) Characterisation of the pig acute phase protein response to road transport. Vet J 173:669–674

SAS Institute (2005) User’s guide. Version 9. SAS Inst. Inc., Cary, NC

Sekiz, S. S., M. L. Scott, and Nesheim, M. C. (1975). The effect of methionine deficiency on body weight, food and energy utilization in the chick. Poultry Science,54:1184–1188

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.

Shen, Y. B., Ferket, P., Park, I., Malheiros, R. D., & Kim, S. W. (2015). Effects of feed grade-methionine on intestinal redox status, intestinal development, and growth performance of young chickens compared with conventional-methionine. Journal of animal science, 93(6), 2977-2986.

Siegel, H. S. (1995). Stress, strains and resistance. British Poultry Science, 36:3–22

Singh, K. S., and Panda, B. (1992). Poultry Nutrition. Kalyani Publishers, New Delhi, India, pp. 57-61.

Soleimani AF, Zulkifli I, Hair-Bejo M, Omar AR, Raha AR (2012b) The role of heat shock protein 70 in resistance to Salmonella enteritidis in broiler chickens subjected to neonatal feed restriction and thermal stress. PoultSci 91:340–345

Soleimani AF, Zulkifli I, Omar AR, Raha AR (2012a) The relationship between adrenocortical function and hsp70 expression in socially isolated Japanese q u a i l . Comp Biochem Physiol - BiochemMolBiol 161:140–144

Soleimani, A. F., Zulkifli, I., Omar, A. R., & Raha, A. R. (2011). Neonatal feed restriction modulates circulating levels of corticosterone and expression of glucocorticoid receptor and heat shock protein 70 in aged Japanese quail exposed to acute heat stress. Poultry science, 90(7), 1427-1434.

Sutanto W., Rosenfeld P., de Kloet E. R., Levine S. (1996). Long-term effects of neonatal maternal deprivation and ACTH on hippocampal mineralocorticoid and glucocorticoid receptors. Brain Research, 92:156–163.

Swick RA, Creswell DC, Dibner JJ, Ivey FJ (1990). Impact of methionine sources on performance of broilers growing under warm and humid conditions [abstracts]. Poultry Science; 69 (suppl.1):194.

Swick, R. A., & Pierson, E. E. M. (1988). Effect of methionine sources and dietary acidulates on broilers during heat stress. Poultry Science, 68(S1), 208.

Swick, R. A., Creswell, D.C., Dibner, J. J., Ivey. F. J. (1990). Impact of methionine sources on performance of broilers growing under warm and humid conditions. Poultry Science, 69 (S1) : 194.

Tohjo H, Miyoshi F, Uchida E, Niiyama M, Syuto B, Moritsu Y, Ichikawa S, Takeuchi M (1995) Polyacrylamide gel electrophoretic patterns of chicken serum in acute inflammation induced by intramuscular injection of turpentine. Poult Sci 74: 648–655

Workel J. O., Oitzl M. S., Fluttert M., Lesscher H., Karssen A., de Kloet E. R. (2001). Differential and age-dependent effects of maternal deprivation on the hypothalamic–pituitary–adrenal axis of brown Norway rats from youth to senescence. Journal of Neuroendocrinology, 13 :569–580.

Xie H, Huff GR, Huff WE, Balog JM, Holt P, Rath NC (2002) Identification of ovotransferrin as an acute phase protein in chickens. Poult Sci 81:112–120

Zulkifli I, Che Norma MT, Israf DA, Omar AR. (2002). The effect of early-age food restriction on heat shock protein 70 response in heat-stressed female broiler chickens. British Poultry Science, 43:141-145.

Zulkifli I., Siegel H. S., Mashaly M. M., Dunnington E. A., Siegel P.B., (1995). Inhibition of adrenal steroidogenesis, neonatal feed restriction and pituitary-adrenal axis response to subsequent fasting. Gen. Comp. Endocrinol. 97:49–56.

Zulkifli I., Soleimani A. F., Khalil M., Omar A.R. (2011). Inhibition of adrenal steroidogenesis and heat shock protein 70 induction in neonatally feed restricted broiler chickens under heat stress condition. Arch. Geflugelkd, 75: 246-252.

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 , P. Najafi, A. J. Nurfarahin, A. F. Soleimani, S. Kumari, A. Anna Aryani, E. L. O'Reilly and P. D. Eckersall, (2014). Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone. Poultry Science, 93:3112-3118

**Internet references**

CJ CheilJedang, (2014). What is L-Methionine. Retrieved from http://www.cjbio.net/l-methionine/index.php.