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Influence of feeding diets containing different levels of L-arginine on blood plasma characteristics of broilers

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Abstract

A total of 300 one-day old *Ross 308* broiler chicks were used i to examine the effect of supplementing of different levels of arginine to broiler diets on blood biochemistry. The chicks were allocated on 4 treatment groups each of five replicates and each replicate consisted of 15 chicks. The treatment groups were control (without arginine) and treatments T1, T2 and T3 having arginine in the diet at the levels of 0.02, 0.04 and 0.06 %, respectively. The blood plasma traits included in this study were protein, albumen, globulin, glucose, alkaline phosphatase, calcium, uric acid, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The results indicated that adding arginine to the diet of broilers (T1, T2 and T3) resulted in significant increases in blood plasma protein, albumen, globulin, glucose, ALP, and calcium and significant decreases in blood plasma cholesterol, uric acid, AST, and ALT during all periods of the experiment. In conclusion, supplementation of the broiler ration with L-arginine resulted in improvement with respect to blood plasma traits. Therefore, arginine can be used as effective feed additive for enhancing physiological status of broiler chickens.

Keywords: Arginine, blood plasma biochemical characteristics, broilers

Introduction

The amino acid arginine is essential for optimal growth and nitrogen balance in growing animals. Whereas most mature mammals can synthesize arginine to meet their requirements, chickens cannot synthesize arginine, therefore, they are completely dependent on the dietary arginine to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). Moreover, arginine has more benefits and has vast effects when it is added as a supplementary diet. For instance, arginine increases the release of growth hormone (GH), facilitates muscle growth (by inhibiting muscle loss), improves muscle performance and improves glucose uptake into muscle cells and it is required for the transport of the nitrogen used in muscle metabolism (Stevens et al., 2000).

Moreover, arginine improves blood circulation, by stimulating the production of nitric oxide (NO), the endogenous neurotransmitter, which helps to prevent vasoconstriction by relaxing the smooth muscle cells of the blood vessels (Huk et al., 1997). Arginine also helps to prevent abnormal blood clotting by stimulating the production of plasmin, increasing vasodilation and inhibiting the adhesion of monocytes to the endothelium, besides, arginine reduces pulmonary blood pressure and improves blood circulation in pulmonary hypertension syndrome that is known as ascites (Nakaki, 1990; Nagaya, 2001).

Arginine lowers total serum cholesterol levels, and serum low-density lipoprotein (LDL) levels, reduces insulin resistance and improves blood sugar disposal in diabetes (Mohan and Cas, 1998). Furthermore, arginine helps to prevent bacterial and viral diseases and enhances immune system functions and increases the size of the thymus (Abdukalykova and Ruiz-Feria, 2006). Arginine also stimulates the production of helper T-cells by the thymus and restores the production of thymic hormones (Dean, 1999).

Deficiency of arginine impaired production of insulinand glucose and metabolism of liver lipids (Zaho et al., 2009), because it is involved in the production of variety of enzymes and hormones. Arginine facilitates the release of GH and stimulates the pancreas for

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insulin production (Recabaren et al., 1996). It also increases the levels of glucose (Emadi et al., 2010). To the best of our knowledge, the role of arginine in poultry is poorly defined. Therefore this study was designed to investigate the effects of dietary supplementation of arginine on the physiological parameters of broilers.

Materials and Methods

A total of 300 one-day old *Ross - 308* broiler chicks were housed in a well-ventilated room. Feed and water were provided *ad libitum* during the whole period of experiment which lasted for 6 weeks. Two types of diets were used in the experiment. Starter diet was used from one to 20 days of chicks age and then grower diet was used till the end of the experiment (Tables 1 and 2).The chicks were allocated for 4 treatment groups and each treatment was consisted of five replicates with 15 chicks each. The treatment groups were: C, the control group (without any addition of arginine), T1, T2 and T3 groups of arginine added to the diet at levels of 0.02, 0.04 and 0.06%, respectively.

Blood samples from 10 chicks per group were obtained (at week three and six of the experimrnt) by cervical dislocation. Serum was separated by centrifugation (1500 rpm for 10 minutes). The serum parameters include in this study were total protein, albumin, globulin, glucose, alkaline phosphatase (ALP), calcium, uric acid, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). These traits were determined according to Al-Daraji et al. (2008).

Data of experiment were analyzed by one- analysis of variance using XLStat (version 7.5. The significance of differences between means of the treatments was tested by Duncan's multiple range test under the probability ($P<0.05$) (Duncan, 1955).

Results

Total protein, albumin, globulin, glucose, alkaline phosphatase and calcium concentrations at third and sixth weeks were significantly high in T3 groupcompared to the control (Tables 3 and 4). Uric acid, cholesterol, AST and ALT concentrations in all the treated groups were significantly lower compared to the control (Table 5 and 6).

Discussion

The significant increase in blood plasma protein, albumin and globulin concentrations in groups fed diet supplemented with arginine may be attributed to the increase in broilers' body weight (Al-Daraji and Salih,

Table 1: Ingredients percentages and calculated proximate analysis of the starter diets used in the experiment

Ingredients (%)	Control	Treatment 1	Treatment 2	Treatment 3
Yellow Corn	58	58	58	58
Soya bean meal	27	27	27	27
Protein conc.*	9	9	9	9
Wheat	4	3.98	3.96	3.94
Sunflower oil	1.5	1.5	1.5	1.5
DCP**	0.3	0.3	0.3	0.3
Salt	0.2	0.2	0.2	0.2
Arginine	0	0.02	0.04	0.06
Total	100	100	100	100
Calculated proximate analysis (% of DM)***				
Protein	22.01	22.00	22.00	22.00
ME, Kcal/Kg DM	3045	3040	3040	3040
Calcium	0.74	0.74	0.74	0.74
Phosphorus	0.41	0.41	0.41	0.41
Lys.	1.3	1.3	1.3	1.3
Meth.	0.62	0.62	0.62	0.62
Meth. to Cyst	0.96	0.96	0.96	0.96
Arginine	1.55	1.57	1.59	1.61
Arg:Lys	1.19:1	1.20:1	1.22:1	1.23:1

* Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40 % crude protein, 2100 Kcal ME / Kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, %3.85 lysine, 3.70 % methionine, and 4% cystine; ** DCP: Dicalcium phosphate; *** The calculated proximate analysis of the diets was determined according to NRC (1994).

Table 2: Ingredients percentages and calculated proximate analysis of the grower diets used in the experiment

Ingrdients (%)	Control	Treatment 1	Treatment 2	Treatment 3
Yellow Corn	61	61	61	61
Soya bean meal	24	24	24	24
Protein conc.*	7	7	7	7
Wheat	5	4.98	4.96	4.94
Sunflower oil	2.5	2.5	2.5	2.5
DCP**	0.3	0.3	0.3	0.3
Salt	0.2	0.2	0.2	0.2
Arginine	0	0.02	0.04	0.06
Total	100	100	100	100
Calculated proximate analysis (% of DM)***				
Protein	20.15	20.13	20.13	20.13
ME, kcal/ KgDM	3150	3145	3145	3145
Calcium	0.6	0.6	0.6	0.6
Phosphorus	0.35	0.35	0.35	0.35
Lys.	1.16	1.16	1.16	1.16
Me.	0.54	0.54	0.54	0.54
Meth. to Cyst.	0.85	0.85	0.85	0.85
Arginine	1.387	1.407	1.427	1.447
Arg:Lys	1.19:1	1.21:1	1.23:1	1.24:1

*Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40 % crude protein, 2100 Kcal ME / Kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, %3.85 lysine, 3.70 % methionine, and 4% cystine; **DCP: Dicalcium phosphate; ***The calculated proximate analysis of the diets was determined according to NRC (1994).

Table 3: Effect of dietary arginine on total protein, albumin and globulin concentrations (Mean ± SE) of broilers at the tested weeks of age

Treatments	Total protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)		
	Weeks		average	Weeks		average	Weeks		average
	3	6		3	6		3	6	
C	2.25 ± 0.07^{d}	3.00 ± 0.48^{d}	2.63 ±0.27^{d}	1.00 ±0.01^{b}	1.00 ±0.10^{b}	1.00 ±0.05^{c}	1.24 ±0.04^{d}	2.00 ±0.29^{d}	1.62 ±0.16^{d}
T1	2.64 ± 0.11^{c}	3.26 ± 0.16^{c}	3.08 ± 0.13^{c}	1.14 ±0.02^{a}	1.16 ±0.02^{a}	1.15 ±0.02^{b}	1.50 ±0.06^{c}	2.39 ±0.09^{c}	1.94 ±0.07^{c}
T2	3.00 ± 0.10^{b}	3.82 ± 0.14^{b}	3.41 ±0.12^{b}	1.17 ±0.04^{a}	1.17 ±0.01^{a}	1.17 ±0.02^{ab}	1.83 ±0.07^{b}	2.6 4±0.07^{b}	2.23 ±0.07^{b}
T3	a 3.84 ± .03^{b}	4.93 ± 0.08^{a}	4.39±0.05^{a}	1.18 ±0.03^{a}	1.19 ±0.02^{a}	1.18 ±0.02^{a}	2.65 ±0.03^{a}	3.74 ±0.05^{a}	3.19 ±0.03^{a}

C: control group; T1, T2 and T3: groups of arginine added to the diet at levels of 0.02, 0.04 and 0.06 %, respectively; a, b, c: Means on the same coulmn having different superscript differ significantly ($p<0.05$).

Table 4: Effect of dietary arginine on glucose and calcium concentrations and alkaline phosphatase activity (Mean ± SE) of broilers at the tested weeks of age

Treatments	Glucose (mg/dl)			Alkaline phosphatase (King Armstrong unit)			Calcium (mg/dl)		
	Weeks		average	Weeks		average	Weeks		average
	3	6		3	6		3	6	
C	135.04 ±5.64^{b}	153.96 ± 9.69^{b}	144.5 ± 7.66^{b}	3382.60 ± 80.49^{d}	1283.55 ± 24.87^{d}	2333.07 ± 32.95^{d}	8.81 ± 0.40^{c}	9.40 ± 0.27^{c}	9.11 ± 0.34^{c}
T1	165.46 ±5.32^{a}	a 184.24 ± 7.34^{a}	174.85 ± 6.33^{a}	3612.52 ± 94.10^{c}	1436.36 ± 7.99^{c}	2524.44 ± 50.13^{c}	10.62 ± 0.57^{b}	11.70 ± 0.27^{b}	11.16 ± 0.36^{b}
T2	166.8 ± 6.93^{a}	186.52 ± 8.02^{a}	176.66 ± 7.47^{a}	4663.88 ± 50.01^{b}	1838.07 ± 18.90^{b}	3250.97 ± 303^{b}	11.23 ± 0.3^{a}	12.56 ± 0.21^{a}	11.90 ± 0.2555^{a}
T3	168.21 ± 5.86^{a}	187.56 ± 7.52^{a}	177.88 ± 6.69^{a}	5331.42 ± 29.18^{a}	2053.87 ± 16.65^{a}	3692.64 ± 16.84^{a}	11.68 ± 0.45^{a}	13.26 ± 0.30^{a}	12.47 ± 0.376^{a}

C: control group; T1, T2 and T3 groups: adding arginine to the diet of broilers at levels of 0.02, 0.04 and 0.06 %, respectively. a, b, c: Means on the same column having different superscript differ significantly ($p<0.05$).

Table 5: Effect of dietary arginine on uric acid and cholesterol concentrations (Mean ± SE) of broilers at the tested weeks of age

Treatments	Uric acid (mg/dl)			Cholesterol (mg/dl)		
	Weeks		average	Weeks		average
	3	6		3	6	
C	5.02 ± 0.439^{a}	4.98 ± 0.246^{a}	5.0 ± 0.3425^{a}	96.74 ± 6.85^{a}	93.60 ± 6.45^{a}	95.17 ± 6.65^{a}
T1	4.83 ± 0.45^{b}	4.80 ± 0.216^{b}	4.91± 0.333^{b}	85.00 ± 8.64^{b}	79.92 ± 8.84^{b}	82.46 ± 8.74^{b}
T2	4.89 ± 0.548^{b}	4.75 ± 0.181^{b}	4.82 ± 0.3645^{b}	92.78 ± 8.24^{b}	79.5 ± 9.07^{b}	86.14 ± 8.655^{b}
T3	4.78 ± 0.353^{b}	4.72 ± 0.244^{b}	4. 75 ± 0.2985^{b}	91.78 ± 9.91^{b}	77.32 ± 6.06^{b}	84.55 ± 7.985^{b}

C: control group; T1, T2 and T3: groups of arginine added to the diet of broilers at levels of 0.02, 0.04 and 0.06 %, respectively; a, b, c: Mean on the same column having different superscript differ significantly ($p<0.05$).

Table 6: Effect of dietary arginine on AST and ALT activity (Mean ± SE) of broilers at the tested weeks of age

Treatments	AST (U / L)			ALT (U / L)		
	Weeks		average	Weeks		average
	3	6		3	6	
C	122.4 ± 4.34^{a}	143.4 ± 3.53^{a}	132.9 ± 3.93^{a}	37.20 ± 4.8^{a}	33.40 ± 3.5^{a}	35.3 ± 1.15^{a}
T1	109.6 ± 4.76^{b}	127.6 ± 2.54^{b}	118.6 ± 3.65^{b}	26.20 ± 8.2^{b}	21.60 ± 6.5^{b}	23.9 ± 2.0^{b}
T2	107.0 ± 9.63^{b}	128.0 ± 3.0^{b}	117.5 ± 6.31^{b}	25.20 ± 5.6^{b}	21.00 ± 7.2^{b}	23.1±1.6^{b}
T3	103.40 ± 14.98^{b}	124.2 ± 3.39^{b}	113.8 ± 9.18^{b}	23.00 ± 7.4^{b}	20.20 ± 6.2^{b}	21.6 ± 1.9^{b}

C: control group; T1, T2 and T3: groups of arginine added at levels of 0.02, 0.04 and 0.06 %, respectively; a, b, c: Means on the same column having different superscript differ significantly ($p<0.05$).

2012a). Heavy body weight was observed to have a high concentration of blood plasma total protein (Corzo et al., 2005). Dietary arginine can contribute to a hormonal environment that increases protein synthesis and muscle growth (Leveilla et al., 1960).

Compounds including creatine, polyamines, praline, glutamate and arginine play a pivotal role in many bodily functions, including protein synthesis (Beaumier et al., 1996). L-arginine has anticatabolic properties because it improves nitrogen balance (Saito et al., 1987). In the current study, arginine decreased H/L ratio via decreasing the effects of glucocorticoids, since glucocorticoid hormone stimulates gluconeogenesis, particularly in the liver (Al-Daraji and Salih, 2012b). This pathway results in the synthesis of glucose from non-hexose substrates such as amino

acids (Wingfield et al., 1998). However, a major part of this increase in protein level in the blood plasma of the arginine supplemented group can be explained by the increase of albumin concentration. Blood plasma or serum electrophoresis showed that albumin is the major part of the total protein in the avian plasma or serum (Al-Daraji et al., 2008).

In the present study arginine supplementation to broilers' diet significantly increased the plasma concentration of glucose. The probable reason for this increase is that arginine stimulates the pancreas for insulin production (Balch et al., 1997). Arginine was reported also to increase the levels of glucose, GH, and glucagon (Braverman, 1997). However, glucagon responses to arginine presence act via different mechanisms on the alpha cell (Reference????). Most obvious was the fact that arginine stimulated glucagon secretion. It is possible, that glucagon responses to arginine may be representative of pancreatic alpha cell responses to amino acids in general. The effects of arginine on glucagon secretion are employing the perfused pancreas technique which indicates that the pancreatic alpha cell is quite responsive to arginine (Pagliara et al., 1973). In this study, arginine supplementation also increased plasma ALP activity and calcium concentration. This may be account for that arginine is involved in remedy of osteoporosis and fractures or bone defects (Civitelli et al., 1992). Arginine is involved in the synthesis of polyamine and L-proline (that act as substrates for collagen synthesis) (Colao et al., 1999), of GH and IGF-I (Chevalley et al., 1998), and in NO (Trippel, 1998) production. NO has been shown to prevent the corticosteroid-induced loss of bone in mature rats (Wimalawansa et al., 1997) and arginine increases ALP activity in late period laying Japanese quail (Onderci et al., 2006). In general, high ALP activity is associated with early cell proliferation, followed by tissue-specific patterns of activity. The possibility that serum ALP activity might serve as a biochemical index of subsequent gain was based on the observation of Li et al. (1947). They reported that GH treatment increased the phosphatase level and that hypophysectomy was followed by a decline in phosphatase.

It was noticed in the present study that arginine lowered plasma cholesterol. This result is in agreement with that of Rossitch et al. (1991) who found arginine to lower serum cholesterol, LDL, and triglyceride levels. Arginine increases the secretion of GH and as a result thyroxine secretion increases; an inverse correlation between serum levels of cholesterol and thyroid hormone has been widely known (Kühn et al., 1993).

In the present study it was shown that adding arginine to broiler diet significantly decreased plasma AST and ALP activity as compared to control group. The decrease in the activity of these two enzymes in blood plasma may be attributed to that arginine helps in detoxification of liver and alleviates cirrhosis by neutralizing ammonia and may be beneficial in the treatment of liver disorders (Braverman, 1997; Balch et al., 1997). So liver malfunction can occur as a result of arginine deficiency (Moss, 1992).

Conclusion

In conclusion feeding diets containing L-arginine resulted in significant improvement in blood plasma parameters levels. Therefore, arginine could be used as an efficient feed additive for enhancing physiological performance of birds.

References

Abdukalykova, A. and Ruiz-Feria, C.A. 2006. Arginine and vitamin E improve the cellular and humoral immune response of broiler chickens. *International Journal of Poultry Science*, 5 (2): 121-127.

Al-Daraji, H.J., Al–Hayani, W.K. and Al–Hassani, A.S. 2008. Avian Hematology. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.

Al-Daraji, H.J .and Atta. S.M. 2012a. Effect of dietary L-arginine on productive performance of broiler chickens. *Pakistan Journal of Nutrition*, 11 (In press).

Al-Daraji, H.J. and Atta. S.M. 2012b. The influence of dietary arginine supplementation on blood traits of broiler chickens. *Pakistan Journal of Nutrition*, 11 (In press).

Balch, M.D., James, F. Balch, C.N. and Phyllis, A. 1997. Prescription for Nutritional Healing. 2nd (Ed.), Avery Publishing Group, New York, Pp: 35-36.

Beaumier, L., Castillo, L., Yu, Y., Ajami, A. and Young. V. 1996. Arginine: new and exciting developments for an “old” amino acid. *Biomedical Environmental Science*, 9: 296-315.

Braverman, M.D. 1997. The healing Nutrients within New Canaan. Keats Publishing, Inc., USA. Pp: 18-23.

Chevalley, T., Rizzoli, R., Manen, D., Caverzasio, J. and Bonjour, J. 1998. Arginine increases insulin-like growth factor-I production and collagen synthesis in osteoblast-like cells. *Bone*, 23: 103-109.

Civitelli, R., Villareal, D., Agnusdei, D., Nardi, P., Avioli, L. and Gennari, C. 1992. Dietary L-lysine and calcium metabolism in humans. *Nutrition*, 8: 400- 405.

Colao, A., Di Somma, C., Pivonello, R., Loche, S., Aimaretti, G. and Cerbone, G. 1999. Bone loss is correlated to the severity of growth hormone

deficiency in adult patients with hypopituitarism. *Journal of Clinical and Endocrinological Metabolism*, 84: 1919-1924.

Corzo, A., Moran, E.T., Hoehler, D. and Lemme, A. 2005. Dietary tryptophan need of broiler males from 42-56 days of age. *Poultry Science*, 84: 226-231.

Dean, W. 1999. The neuroendocrine theory of aging part IV: the immune homeostat. *Vitamin Research News*, 13(10): 1-11.

Duncan, D.B. 1955. Multiple range and multiple F – test. *Biometrics*, 11: 1–42.

Emadi, M., Kaveh, M., Bejo, H., Ideris, A., Jahanshiri , F. Ivan, M. and Alimon, R. 2010. Growth performance and blood parameters as influenced by different levels of dietary arginine in broiler chickens. *Journal of Animal and Veterinary Advances*, 9 (1): 70-76.

Huk, L., Nanobashvili, J., Neumayer, Punz, A., Mueller, M., Afkhampour, K., Mittlboeck, M., Losert, U., Polterauer, P., Roth, E., Patton, S. and Malinski, T. 1997. L-arginine treatment alters the kinetics of nitric oxide and superoxide release and reduces ischemical reperfusion injury in skeletal muscle. Circulation, 96: 667-675.

Kühn, E.R., Berghman, L.R., Moons, L., Vanesande, F., Decuypere, E. and Dores, M. 1993. Hypothalamic and peripheral control of thyroid function during the life of the chicken. In: Avian Endocrinology. Sharp, P.J., (Ed.) P: 29-46. Leveilla, G.A., Feigenbaum, A.S. and Fisher, H. 1960. The effect of dietary protein, fat and cholesterol on plasma cholesterol and serum protein components of the growing chick. *Archives of Biochemistry and Biophysics*, 86: 67-67.

Li, C.H., Kalman C. and Evans, M. 1947. The effect of the hypophyseal growth hormone on the alkaline phosphatase of rat plasma. *Journal of biological chemistry*, 169: 625-630.

Mohan, I. and Cas, U. 1998. Effects of L-arginine-nitric oxide system on chemical induced diabetes mellitus. *Free Radical Biology Medicine*, 25 (7): 757-765.

Nagaya, N. 2001. Short-term oral administration of L-arginine improves hemodynamics and exercise capacity in patients with precapillary pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*, 163 (4): 887-891.

Nakaki, T. 1990. L-arginine induced hypotension. *Lancet*, 336 (8721): 1016-1017.

NRC, National Research Council. 1994. Nutrient Requirements of Poultry. 9th (rev. ed.) National Academic Science. Washington, DC.

Onderci, M., Sahin, N., Sahin, K., Balci, T., Gursu, M., Juturu, V. and Kucuk, O. 2006. Dietary arginine silicate inositol complex during the late laying period of quail at different environmental temperatures. *British Poultry Science*, 47(2): 209-215.

Pagliara, A., Stillings, S., Hover, B. and Matschinsky, F. 1973. Regulation of glucagon and insulin secretion by glucose (G) and amino acids (AA) in the isolated perfused rat pancreas. *Clinical Research*, 22: 847. (Abstr.).

Recabaren, S.E., Jofre, A., Lpbos, A. and Parillo, J. 1996. Effect of arginine and ornithine infusion of leutenizing hormone secretion in prepubertal ewes. *Journal of Animal Science*, 72:162-166.

Rossitch, E. J. 1991. L-arginine normalizes endothelial function in cerebral vessels from hypercholesterolemic rabbits. *Journal of Clinical Investigation*, 87 (4): 1295-1299.

Saito, H., Trocki, O., Wang, S., Gonce, S., Joffe, S. and Alexander, J. 1987. Metabolic and immune effects of dietary arginine supplementation after burn. *Archives of Surgery*, 122: 784-789.

Stevens, B., Godfrey, M., Kaminski, T. and Braith, R. 2000. High intensity dynamic human muscle performance enhanced by a metabolic intervention. *Medicine and Science in Sports and Exercise*, 32 (12): 2102-2104.

Tamir, H. and Ratner, S. 1963. Enzymes of arginine metabolism in chicks. *Archives of Physiology and Biochemistry*, 102: 249-269.

Trippel, S. B. 1998. Potential role of insulin-like growth factors in fracture healing. *Clinical Orthopaedics*, 355: S301-S313.

Wimalawansa, S.J., Chapa, M.T., Yallampalli, C., Zhang, R. and Simmons, D.J. 1997. Prevention of corticosteroid-induced bone loss with nitric oxide donor nitroglycerin in male rats. *Bone*, 21: 275–280.

Wingfield, J.C., Maney, D.L., Breunerj, C.W., Jacobss, D., Lynn, M. Ramenofsky and Richardson, R.D. 1998. Ecological bases of hormone behaviour interactions: the "emergency life history stage". *American Zoology*, 38: 191-206.

Zhao, J.P., H.C. Jiao., Z.G. Song., H. Lin. 2009. Effect of L-arginine supplementation on glucose and nitric oxide (NO) level and activity of NO synthase in corticosterone-challenged broiler chickens (*Gallus gallus domesticus*). *Comparative Biochemistry and Physiology*, Part 150:474-480.