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PHYSIO-BIOLOGICAL MARKERS IN ZINC-INDUCED MOULTED AND NON-MOULTED WHITE LEGHORN LAYERS (*GALLUS DOMESTICUS*)

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ABSTRACT

Various approaches for the induction of moulting have been explained in the literature review, however, there is a dire need to explore other effective methods that can be more efficient in terms of production performance. The current study was conducted to evaluate the role of zinc-induced moulting on certain body performance attributes, serum biochemical profile and serum hormonal profile of White Leghorn layers towards the end of their first production cycle (spent layers). Commercial White Leghorn birds (n=24) of 67-weeks of age were procured from local market and were induced to moult with zinc oxide (3 g/kg diet) for three consecutive weeks. The spent layers were grouped as non-moulted group (NML) and moulted group (ML) after zinc-induced moulting. The result on overall body performance attributes indicated that the body weight and weight of organs significantly ($P \leq 0.05$) decreased in ML group birds as compared to the birds of NML group except for kidneys. The cholesterol, HDL, LDL and triglycerides were lower ($P \leq 0.05$) in birds of ML group as compared to birds of NML. On the contrary, serum circulating levels of ALT and AST were statistically higher ($P \leq 0.05$) in birds of ML group as compared to their counterpart NML group. A significant increase ($P \leq 0.05$) was noticed in serum T4 and cortisol concentrations for birds of ML group as compared to those of NML group, whereas T3 level was found decreased ($P \leq 0.05$) in birds of ML group. The results of present study indicated that usage of dietary supplementation of ZnO (3g/kg), for the purpose of moulting, exhibits promising effects on body performance attributes and serum biomarkers. Different dietary concentrations of ZnO may be evaluated in future to minimize stress and further enhance the performance of spent layers.

Keywords: Cortisol, Moulting, Spent layers, Zinc-oxide

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INTRODUCTION

Moulting is a natural process for recycling production cycle in all species of birds. Induced moulting has become an important managemental tool in commercial poultry industry to get better and prolonged egg production in laying hens during their subsequent production cycles (Anwar *et al.,* 2018). For this purpose, various techniques have been implied including water and food deprivation alone or both namely 'fast-induced moulting, dietary manipulation of certain nutrients, and hormone-induced moulting. Fast-induced moulting has long been used for inducing moult in spent layers (Huo *et al.,* 2020) but this method of moulting has been criticized by advocates of animal welfare due to possible outcome of stress (Sandhu *et al.*, 2010; Anwar *et al.*, 2018). Among various dietary manipulation techniques, zinc-induced moulting has been reported to exhibit beneficial effects on laying hens in terms of better post-moult performance and better dynamics of pituitary-hormone-producing cells (Sandhu *et al.,* 2010). Zinc is an integral part of over 200 metalloenzymes. Higher dietary concentrations have the potential to reduce the susceptibility of salmonella enteritidis, enhance egg production and lower mortality along with intensification of circulating serum hormones (Idowu *et al.,* 2011, Park *et al.,* 2018). The zinc-induced moulting method has been less criticized, reported to be less stressful and superior to forced moulting by many researchers and the upholders of poultry welfare (Silva-Mendonça *et al*., 2015).

Effects of various supplementations for alleviating stress after zinc-induced moulting on overall production parameters, eggshell quality, immunity, mineral deposition, and immunochemistry of pituitary at various stages of production during second and third egg-laying cycle of spent layers have already been reported by many researchers (Glatz *et al.,* 2020). However, the possible role of zinc-induced moulting at higher levels of zinc supplementation on performance and certain serum biological biomarkers of White Leghorn Layers still needs to be evaluated (Kakhki *et al*., 2018; Fard *et al.,* 2020). Therefore, keeping in view the significant role of zinc supplementation on well-being of White Leghorn

Layers the present research was designed to investigate the effect of zinc-induced moulting on body weight and organ weight, serum biochemical profile (high-density lipoproteins, low-density lipoproteins, triglycerides, alanine aminotransferase and aspartate aminotransferase), and serum hormonal profile (triiodothyronine, thyroxine and cortisol) of spent laying hens.

MATERIALS AND METHODS

Experiment Design: The experiment was aimed to evaluate the impact of zinc-induced (Zinc oxide) moulting on body performance attributes and serum biological markers in White Leghorn layers *(Gallus domesticus)*. Commercial cage-housed White Leghorns layers (*Gallus gallus domesticus*) (n=24) towards the end of first production cycle (67 weeks old) were obtained from commercial layer farm. The birds were reared during February to March 2011 at Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad, Pakistan. The study was approved by the Institutional Research Biosafety and Bioethics Committee, University of Agriculture, Faisalabad, Pakistan (No. 14388-91).

Table 1: Ingredients and composition of basal feed ration (g/100g of diet).

Ingredients	Inclusion level (%)
Corn	40
Rice Tips	10
Maize Gluten 30 %	6
Soyabean Meal	8
Fish Meal	6
Rice Polishing	11
Limestone Powder	7
Canola Meal	10
D.C.P	1.5
Vitamin mineral Premix[a]+ Amino acid[b]	0.5
Total	100
Nutritive Value	
Crude Protein	16
Energy	2795 Kcal

[a]Premix / kg feed: Cholecalciferol 2200 U; Riboflavin 5.5 mg; Vitamin A 8300 U; D-calcium pantothenic acid 15 mg; Niacin 36 mg; Choline 500 mg; Folic acid 0.5 mg; Vitamin B1 1 mg; Pyridoxine 2.2 mg; Biotin 0.05 mg; Vitamin K 2 mg; Vitamin E 8 U; Vitamin B12 0.02 mg; Manganese 80 mg; Zinc 60 mg; Iron 60 mg; Copper 5 mg; Cobalt 0.2 mg; Iodine 1 mg; Selenium 0.15 mg; [b]Amino Acids/kg feed: Lysine 0.72 g; Methionine, 0.143 g; Threonine, 0.35 g.

The birds were acclimatized in cage-housing having one bird per cage with provision of basal feed ration 100 g/day/bird (Table 1), 16 hours light duration and ad-libitum water. At the end of acclimatization, randomly selected birds (n=12) were humanely slaughtered for the collection of blood and tissue samples and were grouped as non-moulted layers (NML). The remaining birds (n=12) were induced to moult using 3g/kg dietary ZnO for three weeks as described earlier (Anwar *et al.,* 2018) and were grouped as moulted layers (ML). Briefly, during the moulting period of three consecutive weeks, the birds were provided with light duration of 12 hours and layer ration was offered at 35 g/bird per day. At the end of moulting phase, moulted layers (n=12) were slaughtered for the collection of organs, and blood samples. Blood samples were collected and centrifuged (4000 rpm) for 10 minutes and harvested sera were stored at -20ºC till further analyses.

Body Performance Attributes: The overall body weight of birds and their organs (pituitary, brain, spleen, liver, heart and kidney) in both NML and ML groups were recorded at the time of each sampling when all were slaughtered to collect blood and tissue samples.

Serum Biochemical Profile: Colorimetric estimation of serum total cholesterol and serum high-density lipoprotein (HDL) was conducted by using commercially available kits (CAT# 4248; Biocon®, Diagnosemittel Gmbh, Hecke 8, 34516Vohl-Marienhagen, Germany). The concentration of serum low-density lipoproteins (LDL) was determined by subtracting concentrations of HDL from total cholesterol concentration.

Estimation of triglycerides (TGs) in the serum sample was done using commercially available kit (CAT# 10724; Human Diagnostica mbH, Wiesbaden-Germany) using the same formula as mentioned above for total cholesterol calculation.

Serum Alanine Aminotransferase (ALT) level in the sample was determined by using the commercially available ELISA based kit (CAT# AL146; Randox Laboratories Ltd., UK). Similarly, serum Aspartate Aminotransferase (AST) level was estimated by using commercially available ELISA kit (Ref # AS147; Randox Laboratories Ltd., Crumlin, Co. Antrim. UK).

Serum Hormonal Profile: The serum concentration of triiodothyronine (T3) (CAT# JT003; JD Biotech®, Italy), thyroxine (T4) (µg/dL; Ref # JT005; JD Biotech®, Italy) and cortisol (CAT# 3625-300; Monobind Inc. USA) were determined using commercially available ELISA kits.

Statistical Analysis: Statistical analysis was performed using Statistical Package for Social Sciences (V.17, SPSS Inc., Chicago, IL, USA). The results were mentioned as mean ± SE. Difference between the two groups *viz*. NML and ML was deduced using independent t-test.

RESULTS AND DISCUSSION

Body Performance Attributes: The result on overall body performance revealed that the body weight and weight of organs significantly (P≤0.05) decreased in ML group birds as compared to the birds of NML group except for kidneys (Table 2). The dietary zinc up to 1000 mg/kg diet has been reported to impart no adverse effect on the body weight of hens, however, inclusion of excessive dose of zinc (3 g/kg) in the diet of hens has been found to decrease the body weight of laying hens (Kim and Patterson, 2005) which is in compliance to our results. The decline in the relative weight of liver, brain, pituitary and heart while increase in the relative weight of kidney has been reported in the birds moulted by supplementation of high dietary zinc as compared with birds induced to moult by fasting in an studies (El-Gendi *et al.,* 2009; Abdullah, 2007). The higher kidney weight in zinc supplemented birds might be due to zinc accumulation (El-Gendi *et al.,* 2009). El-Deek and Al-Harthi (2004) also have reported a decline in body and organ weights of laying hens induced to moult by dietary zinc oxide.

Table 2. Overall mean (± SE) values for body performance attributes, serum biochemical and hormonal profile in non-moulted and zinc-induced moulted layers.

Parameters	NML	ML
Body weight (Kg)	1.52±0.02	0.99±0.03*
Pituitary (mg)	12±0.4	4.8±0.4*
Brain (g)	2.77±0.02	2.64±0.04*
Spleen (g)	1.4±0.08	0.44±0.03*
Liver (g)	37.97±1.55	12.8±0.57*
Heart (g)	6.93±0.15	4.28±0.21*
Kidney (g)	4.41±0.34	6.49±0.36*
Serum Biochemical Profile		
Cholesterol (mg/dL)	150.48±5.75	120.23±5.1*
HDL-Cholesterol (mg/dL)	62.47±5.2	47.86±2.86*
LDL-Cholesterol (mg/dL)	88.11±7.2	69.39±5.24*
Triglycerides (mg/dL)	170.97±7.37	128.75±9.99*
Alanine aminotransferase (Unit/L)	34.59±0.77	53.67±1.73*
Aspartate aminotransferase (Unit/L)	50.66±3.97	200.89±4.9*
Serum Hormonal Profile		
Triiodothyronine (ng/mL)	6.52±0.46	1.17±0.09*
Thyroxine (µg/dL)	12.88±0.44	16.28±0.92*
Cortisol (µg/dL)	2.87±0.2	5.77±0.13*

NML= Non-moulted layers; ML= Moulted layers; *Significant at (P≤0.05)

It has been established that the suppression of feed consumption after inclusion of high dietary zinc leads to reduction in body weight, and egg production of laying hens. (Kim and Patterson, 2005; Park *et al.,* 2004a) reported that compared to non-moulted layers the moulted layer hens that were induced to moult by Zn-propionate and Zn-acetate showed a reduction in their dietary intake up to 65 % and 70 %, respectively. Similarly, another study (Kim and Patterson, 2005) showed that hens fed the diet supplemented with $ZnSO_4$ at 2000 ppm and 3000 ppm had reduced feed consumption resulting in ultimate reduction of body weight and egg production. Yet another report (Reddy *et al.,* 2008) reports that Cornish layers lost about 16% and Rock layers about 21.7% body weight respectively, due to zinc-induced moulting (25,000 pm), however, a reduction in the body weight up to 35% was observed in this study.

The reduced feed intake could be attributed to suppression of appetite centre in layers due to higher dietary zinc (Park *et al.,* 2004b) resulting in lack of proper nutrient availability and reduction in the muscle mass of spent layers (El-Deek and Al-Harthi, 2004), which ultimately decreases the body and organs weights of the birds (Neto *et al.,* 2020).

Serum Biochemical Profile: In current study, the concentrations of serum cholesterol, HDL, LDL and TGs were significantly decreased (P≤0.05) in zinc-induced moulted layers (Table 2) as compared to their counterpart NML group. In laying hens, dietary inclusion of zinc (75 mg ZnO/kg diet) resulted in decreased (P≤0.001) serum concentrations of TGs and LDL level (Abd El-Hack *et al.,* 2020). Similarly, dietary inclusion of zinc in broiler birds resulted in a reduction in the concentration of total lipids, cholesterol, HDL and LDL than control birds (EL-Gogary and Abo EL-Maaty 2020). The literature also demonstrated a decline in serum cholesterol and HDL after supplementation of excessive dietary zinc in chicks (Abd El-Hack *et al.,* 2018). The decline in the concentration of serum TGs has been observed after feeding zinc-oxide (200 mg/kg) in the diet of laying hens (Jaensch *et al.*, 2000; Kaya *et al.,* 2001). The effect of zinc supplementation on lipid profile hinders lipolysis induced by adrenaline and enhances the lipogenic activities in the rat adipocytes (Lutosławska and Fornal-Urban, 2009). The decline in lipid profile in the present study might be due to the utilization of body fat reserves from the adipocytes as a result of lower feed intake after zinc supplementation (Lutosławska and Fornal-Urban, 2009).

Serum circulating levels of ALT and AST in the present study were statistically higher ($P \leq 0.05$) in birds of ML group as compared to their counterpart NML group (Table 2). Increased activity of plasma AST and ALT is an indicative of organ damage, specifically liver. These serum enzymes usually are indicators of oxidative damage to the liver tissues as elaborated earlier for birds (Che *et al.,* 2010; Surai, 2002). The enzyme activity of AST is considered nonspecific but sensitive indicator of liver disease (Jaensch *et al.,* 2000).

Hussain *et al.* (2019) suggested elevated level of serum AST to be related with the gluconeogenesis in liver. The enhanced AST and ALT level may be due to moulting-induced stress which increases adrenal activity and corticosterone stimulation, adrenal activity has direct correlation with ALT concentration. The higher activity of the serum AST and ALT can be attributed due to the increased oxidative damage in the hepatic tissue of spent layers after excessive dietary zinc in moulted layers. Moreover, AST level also varies with genomic and climatic variation (Hassaan *et al., 2009*). However, no obvious difference in plasma ALT or AST level between layers fed different doses of dietary zinc supplementation (Yang *et al.,* 2004; EL-Gogary and Abo EL-Maaty, 2020).

Serum Hormonal Profile: In the present study, serum T4 was higher ($P \leq 0.05$) for birds of ML group (zinc-induced moulted), whereas T3 level decreased significantly ($P \leq 0.05$) in birds of ML group (Table 2). Thyroid glands produce hormones which are derived from the tyrosine and these hormones, T_3 and T_4 are involved in the regulation of body metabolism. The Triiodothyronine has a role in thermoregulation of body and is a better indicator of heat production than T_4 serum concentration. Zinc-oxide enhancement in the diet has shown to increase thyroid hormones in broilers (EL-Gogary and Abo EL-Maaty 2020). The increase in serum T_4 concentration and decrease in serum T_3 concentration has been reported in zinc-induced (1 % zinc oxide) moulted layers earlier (El-Gendi *et al.,* 2009). The lower serum concentration of T_3 after zinc supplementation might be due to low feed intake by birds (Buyse *et al.,* 2002). Elevated serum T_4 and decreased T_3 concentration in zinc-induced moulted layers might be attributed to the decreased de-iodination which inhibits the conversion of prohormone thyroxine (T_4) to the physiologically active form T_3 in tissues (Elnagar and Bech, 2000; Costantini *et al.,* 2008).

Cortisol level in present study was higher for ML group birds (zinc-induced moulted) as compared to their non-moulted counterpart birds. Higher level of circulatory corticosterone reflects the period of stressful conditions in laying hens as depicted earlier (Onbaşılar and Erol, 2007). Elevated level of corticosterone is involved in production of reactive oxygen metabolites leading to the development of oxidative stress (Costantini *et al.,* 2008). In the moulted layers fed zinc-oxide (3000 mg/kg diet), an increased serum cortisol level has been reported towards the end of moulting as compared to the birds before moulting (Sandhu *et al.,* 2010). Zinc feeding leads to reduction in feed intake by spent layers by suppressing the appetite centre (Park *et al.,* 2004a). Increase concentration of circulatory corticosteroids could be due to the stress induced by the poor availability of nutrients after supplementation of zinc-oxide (3 g/kg diet).

Conclusion: In a nutshell, the results of present study reveal that the dietary inclusion of ZnO (3g/kg) for zinc-induced moulting in spent layers did successfully induce moulting with substantial effect on body performance attributes and serum biomarkers. We recommend different levels of ZnO with lower dose rate for induced moulting in order to attain optimal level for decreased stress and increased performance attributes in moulted birds.

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REFERENCES

Abdullah, M. (2007). Immunohistochemistry of pituitary gland and immunological profiles of molte chicken. (Doctoral dissertation University Of Agriculture, Faisalabad Pakistan).

Abd El-Hack, M. E., M. Alagawany, A.S. Salah, M.A. Abdel-Latif and M.F.A. Farghly (2018). Effects of dietary supplementation of zinc oxide and zinc methionine on layer performance, egg quality, and blood serum indices. Biol. Trace. Elem. Res. 184:456–462.

Abd El-Hack, M.E., M. Alagawany, M.T. Chaudhry, M. Saeed, E.A.M. Ahmad and S.A.A. El-Sayed (2020). Does the gradual increase in dietary zinc oxide supplementation can affect egg quality, serum indices, and productive performance of laying hens? Trop. Anim. Health. Prod. 52:525–531.

Anwar, H., Z.ur. Rahman and M. Idris (2018). Dynamics of endocrine markers and liver enzymes in laying hens after protein and probiotics supplementation in the post-moult phase. J. Appl. Anim. Res. 46:977–983.

Buyse, J., K. Janssens, S. Van der Geyten, As.P. Van, E. Decuypere and V.M. Darras (2002). Pre-and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens. Br. J. Nutr. 88:641-653.

Che Z., Y. Liu, H. Wang, H. Zhu, Y. Hou and B. Ding (2010). The protective effects of different mycotoxin adsorbents against blood and liver pathological changes induced by mold-contaminated feed in broilers. Asian Australas J. Anim. Sci. 24:250-257.

Costantini, D., A. Fanfani and G. Dell'Omo (2008). Effects of corticosteroids on oxidative damage and circulating carotenoids in captive adult kestrels (Falco tinnunculus). J. Comp. Physiol. B. 178:829–835.

El-Deek, A. and M. Al-Harthi (2004). Post molt performance parameters of broiler breeder hens associated with molt induced by feed restriction, high dietary zinc and fasting. Int. J. Poult. Sci. 3:456-462.

El-Gendi, G. M., H.R. Samak and A.A. Mohamed (2009). Effect of induced molting on some productive and physiological traits in Hy-Line hens. Egypt. Poult. Sci. J. 29:385–405.

EL-Gogary, M.R. and H.M.A. Abo EL-Maaty (2020). Impact of zinc supplementation and stocking density on performance, physiological and immune responses in broiler chickens. J. Ani. Poult. Pro. 11:95–102.

Elnagar,S.A and M. Bech (2000). Heat stress-induced hypothyroidism mediated changes in reproductive hormones in laying hens. In: proceeding of International Poultry Scientific forum abstracts, concurrent meeting of the Southern Poultry Science Society, the Southern Conf. on avian diseases. January, pp:17-18.

Fard, M. K., R. Ghasemi, E. Dirandeh, M. Torki and M. Rezaei (2020). Effect of zinc oxide, potassium iodide and withdrawal diet as alternative moulting methods on performance of commercial laying hens. J. Appl. Anim. Res. 48:534–542.

Glatz, P. C., A.J. Tilbrook, P.C. Glatz and A.J. Tilbrook (2020). Welfare issues associated with moulting of laying hens. Anim. Prod. Sci. 61:1006–1012.

Hassaan, S. F., S.A. Abdel-fattah, A.E. Elsalmoney and M.S.H. Hassan (2009). Relationship between some serum enzyme activities, liver functions and body weight in growing local chickens. Int. J. Poult. Sci. 8:700-705.

Hussain, Z., J.A. Khan, A. Arshad, P. Asif, H. Rashid and M.I. Arshad (2019). Protective effects of Cinnamomum zeylanicum L. (Darchini) in acetaminophen-induced oxidative stress, hepatotoxicity and nephrotoxicity in mouse model. Biomed. Pharmacother. 109:2285–2292.

Huo, S., Y. Li, Y. Guo, S. Zhang, P. Li and P. Gao (2020). Improving effects of Epimedium flavonoids on the selected reproductive features in layer hens after forced molting. Poult. Sci. 99:2757–2765.

Idowu, O., R. Ajuwon, A. Oso and O. Akinloye (2011). Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. Int. J. Poult. Sci. 10:225-230.

Jaensch, S.M., L. Cullen and S.R. Raidal (2000). Assessment of liver function in Galahs (*Eolophus roseicapillus*) after partial hepatectomy: A comparison of plasma enzyme concentrations, serum bile acid levels, and galactose clearance tests. J. Avian. Med. Surg. 14(3):164–171.

Kakhki, A.M., R., Z. Mousavi and K.E. Anderson (2018). An appraisal of moulting on post-moult egg production and egg weight distribution in white layer hens; meta-analysis. Anim. Prod. Sci. 59:278–285.

Kaya, Ş., H.D. Umucalilar, S. Haliloğlu and H. İpek (2001). Effect of dietary vitamin A and zinc on egg yield and some blood parameters of laying hens. Turk. J. Vet. Anim. Sci. 25:763-769.

Kim, W.K and P.H. Patterson (2005). Effects of dietary zinc supplementation on hen performance, ammonia volatilization, and nitrogen retention in manure. J. Environ. Sci. Health. Part B. 40:675–686.

Lutosławska, G and A. Fornal-Urban (2009). Zinc contribution to the regulation of glucose disposal, lipid metabolism and striated muscle contractions. Med. Sport. 2009; 13: 28-34.

Neto, M.A.T., J.C. Dadalt and M.L.P Tse (2020). Dietary combination of chelated zinc and threonine and effects on egg production, egg quality and nutrient balance of Brown laying hens from 20 to 49 weeks of age. Anim. Feed. Sci. Tech. 267:114555.

Onbaşılar, E and H. Erol (2007). Effects of different forced molting methods on postmolt production, corticosterone level, and immune response to sheep red blood cells in laying hens. J. Appl. Poult. Res.16:529-536.

Park, E., H.D. Paik and S.M. Lee (2018). Combined effects of whey protein hydrolysates and probiotics on oxidative stress induced by an iron-overloaded diet in rats. Int. J. Food Sci. Nutr. 69:298–307.

Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet and S. C. Ricke (2004a). Review on the role of dietary zinc in poultry
nutrition, immunity and reproduction. Biol. Trace Elem. Res. 101: 147-163.

Park, S. Y., W. K. Kim, S. G. Birkhold, L. F. Kubena, D. J. Nisbet and S. C. Ricke (2004b). Using a feed-grade zinc propionate to achieve molt induction in laying hens and retain postmolt egg production and quality. Biol. Trace. Elem. Res. 101:165-179.

Reddy, V.E., V.K. Malathi, V. K and B.S.Venkatarami (2008). Effect of induced moulting in male and female line broiler breeder hens by zinc oxide and feed withdrawal methods on post molt performance parameters. Int. J. Poult. Sci. 7:586-593.

Sandhu, M.A., Z.U. Rahman, A. Riaz, S.U. Rahman, I. Javed and N. Ullah (2010). Somatotrophs and lactotrophs: an immunohistochemical study of Gallus domesticus pituitary gland at different stages of induced moult. Eur. J. Histochem.54(2):e25.

Silva-Mendonça, M.C.A., N.S. Fagundes, G.A. Mendonça, F.C. Gonçalves, B.B. Fonseca, A.V. Mundim (2015). Comparison of moulting methods for layers: high-zinc diet versus fasting. Bri. Poult. Sci. 56:598–604.

Surai, P.F (2002). Natural antioxidants in avian nutrition and reproduction. Nottingham University Press Nottingham.

Yang, L., S. Coa, M. Cheng, L. Chen and k. Chen (2004). Effects of iron, zinc, iodine and selenium levels in rations on activities of metabolic enzymes of layers and egg quality. J Huazhong Agri. Univ. China. 62:113-119