ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.112.117

Review Article
Improved Production Performance and Health Status with Winter Mushroom Stem (*Flammulina velutipes*) in Laying Chicken: Review

[1,2]S.U. Mahfuz, [1,3]Song Hui and [4]Liu Zhongjun

[1]School of Live Science, Jilin Agricultural University, 2888 Changchun, Jilin, People's Republic of China
[2]Department of Animal Nutrition, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, 3100 Sylhet, Bangladesh
[3]Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, 130118 Changchun, China
[4]College of Traditional Chinese Medicine, Jilin Agricultural University, Changchun, Jilin, People's Republic of China

Abstract

This study was conducted to represent the role of *Flammulina velutipes* stem in laying hen ration for improving laying performances and health status of layer chicken. *Flammulina velutipes* is one of the most popular edible mushroom in Asian countries and known as winter mushroom. Inclusion of *Flammulina velutipes* stem (FVS) in layer diets is the most effective supplement in order to improve laying performance, egg quality, intestinal morphology, cecal microflora and excreta composition. It has been reported that FVS in laying hen could improve laying performance and egg quality including Feed Conversion Ratio (FCR), egg mass, egg size, egg yolk cholesterol and yolk fatty acid. *Flammulina velutipes* stem can change small intestinal morphology that might help to increase nutrient absorption in chicken. *Flammulina velutipes* stem also reported on controlling pathogenic bacteria as well as increasing beneficial bacteria in cecum to keep sound gut health in layer chickens. *Flammulina velutipes* stem had indirect effect on suppression of ammonia gas in feces by reducing moisture content in feces. Therefore supplementation of winter mushroom stem could be useful strategy to improve laying performance, egg quality and sound gut health in layer.

Key words: *Flammulina velutipes* stem, laying performance, egg quality, gut health, excreta composition

Received: January 25, 2017 **Accepted:** February 28, 2017 **Published:** March 15, 2017

Citation: S.U. Mahfuz, Song Hui and Liu Zhongjun, 2017. Improved production performance and health status with winter mushroom stem (*Flammulina velutipes*) in laying chicken: review. Int. J. Poult. Sci., 16: 112-117.

Corresponding Authors: S.U. Mahfuz, Department of Animal Nutrition, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, 3100 Sylhet, Bangladesh Tel: +88 01724671408
Song Hui, School of Live Science, Jilin Agricultural University, 2888 Changchun, Jilin, People's Republic of China Tel: +8613604449943

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inclusion of dietary winter mushroom (*Flammulina velutipes*) stem in layer diet can be used for improving laying hen performance and health status including Feed Conversion Ratio (FCR), egg mass, egg quality, cecal microflora, excreta Dry Matter (DM) and excreta ammonia gas emission. *Flammulina velutipes* is the thin white mushroom commonly used in east Asian countries such as China, Japan, Vietnam and Korea. These mushrooms are also known by the name golden needle mushroom or lily mushroom or winter mushroom[1]. Mushrooms are recognized as having considerable health benefits and desirable functions. It also has long been appreciated for their flavor and texture, medicinal and tonic attributes. However, the more recent concept is that mushroom represents an important source of biologically active compounds of medicinal value. Mushrooms are now known to be rich in high quality protein, vitamin, minerals and a high proportion of unsaturated fatty acids[1]. The use of probiotics, products made from living micro-organisms including bacteria, fungi and yeast have been considered a strong substitute to antibiotics because probiotics do not lead to antibiotic-resistant bacteria in the gastrointestinal tract[2]. Anti-inflammatory, anti-tumor and immune-modulating drugs as well as dietary supplements were produced form the mushrooms[3]. Mushroom wastes are widely left from mushroom production industries and have been supposed to possess prebiotic, anti-microbial, anti-fungal and anti-oxidant properties[4]. *Flammulina velutipes*, one of the most popular edible mushrooms has attracted considerable attention of biochemistry and pharmacology due to its biological activities[5]. *Flammulina velutipes* is an excellent source of essential amino acids and vitamins, this fungi also exhibits good anti-oxidant, anti-inflammatory, immunomodulatory, anti-tumor and cholesterol-lowering activities[6,7]. In addition, *F. velutipes* also contains various kinds of bioactive substance such as α-glucan, hetero polysaccharides, mannofucogalactan and monoterpenes, which have anti-carcinogenic and immunomodulating effect[8,9]. Moreover, compounds with medicinal properties have been isolated from the fruiting body and mycelial culture of this mushroom, including proteins with antiviral and immunomodulatory activity[10], polysaccharides with immunomodulatory activity[3], lectin with anti-tumor activity[11], sesquiterpenoids with antimicrobial activity[12,13] and sterol[14]. In the meantime functional polysaccharide is discovered from *F. velutipes* mushroom that may play effective role in poultry production. However, potential use of F*. velutipes* in animal study is still limited.

Since many years researchers are looking for natural unconventional feed resources to improve the animal and poultry production performance. With the removal of antibiotic growth promoters from poultry diets in different areas of the world, it is of interest to investigate potential substitute to maintain optimum performance, intestinal health and microbial populations, particularly to control the growth of harmful bacteria. Due to recent ban of using antibiotic as growth promoters in poultry diet, mushroom wastes could be a proper substitute for antibiotic in poultry ration. *Flammulina velutipes* stem is an agricultural byproduct having nutritional and medicinal values and its availability is abundant but up to now its utilization is limited for animal production. Very few studies have been carried out yet to evaluate the feasibility of *Flammulina velutipes* stem on laying hens. Considering the above facts, this study was focused on the use of *Flammulina velutipes* stem as a feed ingredient as well as a substitute of antibiotics to improve productivity and health status in laying hens.

PROXIMATE ANALYSIS OF *F. velutipes* STEM

The chemical analysis of *F. velutipes* stem showed that Dry Matter (DM) 84-89.6%, Crude Protein (CP) 8.9-11.8%, carbohydrate 43.33-69.40%, Total Detergent Fiber (TDF) 1.9-7.40%, crude fat (EE) 1.79-2.91%, ash (total mineral) 4.91-7.40%, calcium (Ca) 2.21% and phosphorus (P) 1.68% by previously published value[15-18]. The low fat but high in protein as well as fiber is the unique character of *F. velutipes* mushroom. *Flammulina velutipes* is also exhibit excellent sources of amino acids like methionine, valine, isoleucine, leucine, lysine, phenylalanine and threonine those are very important and sensitive nutrient components in poultry diet[19].

EFFECT OF *F. velutipes* STEM SUPPLEMENTATION IN LAYING PERFORMANCE

Few studies have been conducted in previous years to investigate the effect of dietary *F. velutipes* stem supplementation in layer chickens. Lee *et al.*[20] reported that fermented *F. velutipes* mycelium showed positive effect on laying performance parameters including; egg mass, egg size and FCR in laying hens. It was thought that *F. velutipes* stem contain higher level of CP that lead to increase egg albumin and might have effect on shell gland for continuous egg shell formation. Lee *et al.*[18] stated that adding *F. velutipes* mycelium at 5% in layer diet did not affect feed intake but slightly improved growth performance and FCR. Hen mortality was significantly low that was directly

associated with immunity gained from supplemented *F. velutipes* stem as the polysaccharides in *F. velutipes* have immune modulatory activity[1,3,21,22].

EFFECT OF *F. velutipes* STEM SUPPLEMENTATION ON EGG QUALITY

The egg quality parameters including; egg size, shell thickness, egg yolk cholesterol directly and indirectly influence to egg consumers. Dietary supplementation of 4% *F. velutipes* mycelium could improve egg shell weight, shell thickness albumin height and haugh unit[20,23]. In contrast Na *et al.*[17] reported that dietary supplementation of *F. velutipes* media had no effect on egg shell weight and thickness. Sun *et al.*[24] reported that edible mushrooms have hypo-cholesterolemic effect on health and suggested to use it as oral medicine. Yeh *et al.*[25] investigated the effect of the active components of both *F. velutipes* powder and *F. velutipes* extract on the lipid metabolism of male hamsters on a high fat diet. The study revealed that both *F. velutipes* extract and powder at dose of 3% are capable of reducing the level of Total Cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL) and LDL/HDL (high density lipoprotein cholesterol) in the serum and liver of the hamsters significantly. At present considerable interest has arisen in the use of natural antioxidants derived from unconventional resources for improving egg quality by increasing its anti-oxidative potential. Butylated hydroxyanisole and butylated hydroxytoluene are examples of potent synthetic anti-oxidants, however, some of them are found to be toxic and carcinogenic to human body, thus efforts are now focusing on discovering natural anti-oxidant products[26]. It was assumed that *F. velutipes* stem have antioxidant activity helped to reduce oxidation in egg yolk resulted lower thiobarbituric acid value (TBA). The polysaccharide (*Flammulina velutipes* polysaccharide, FVP I-A) has strong anti-oxidant and immunomodulatory value[21]. Phenolic compounds especially phenolic acid is the major naturally occurring anti-oxidant components found in medicinal mushrooms like *F. velutipes*[27,28]. However, there is no evidence as to whether dietary supplementation of dried winter mushrooms can improve oxidative stability of chicken tissues. *Flammulina velutipes* mushroom is also considered as a good source of selenium compounds and probably the selenium of *F. velutipes* improved the oxidative stability of egg yolk[29]. Diet contains rich in total omega-3 fatty acid (n-3), total omega-6 fatty acid (n-6) as well as Poly Unsaturated Fatty Acid (PUFA) is useful for human and animal health. A diet rich in n-3 PUFA is thought to be therapeutic beneficial for people with either cardiovascular disease or rheumatoid arthritis and very useful for their preventative role in a wide range of conditions[30,31]. *Flammulina velutipes* naturally contain smaller quantity of Saturated Fatty Acid (SFA) and Mono Unsaturated Fatty Acid (MUFA) than PUFA[32]. Higher abdominal fat in laying hen can reduce total egg production and cause some metabolic disorders like fatty liver diseases and winter mushroom could be an alternative way to overcome this problem.

EFFECT OF *F. velutipes* STEM ON SMALL INTESTINAL MORPHOLOGY

Chickens intestine morphological characteristics are very important for optimum nutrient utilization as well as for keeping good health. Higher intestinal absorptive and good gut health is associated with the higher villus height and lower crypt depth[4]. The structural improvement of villus height and villus height-crypt depth ratio may be correlated with high content of crude fiber in FV mushroom. Many studies have been suggested that supplementation of mushroom increased the duodenal, jejunal and ileal villus height of the birds[4,33].

EFFECT OF *F. velutipes* STEM ON GUT HEALTH

Gut system affect the way nutrients are partitioned and utilized for organ development, tissue growth and immune system maturation. Cecal microflora populations are indicative of gut health in animals and poultry. Diet has direct and indirect role to keep healthy gut in poultry. Laying hens fed supplemented with *F. velutipes* (3-5%) could reduced the pathogenic bacteria like *Salmonella* spp., *E. coli* and *Clostridium* spp., as well as increased change in concentration of beneficial bacteria of *Lactobacillus* spp., and *Bifidobacterium* spp.,[18,20,34]. *Flammulina velutipes* have immunomodulatory effect via induction of cytokines [22,35]. Guo *et al.*[36] stated that mushroom and herb extract diets reduced *Bacteroides* spp., Enterococci and *E. coli* numbers, but increased numbers of Bifidobacteria and Lactobacilli. Another study by Willis *et al.*[37] stated that Bifidobacteria proliferation is enhanced with the mushroom extract. The polysaccharides from *F. velutipes* mycorrhizae (PFVM) increased the body weight to mice and the weight ratio of the thymus and spleen as a result, the T-cell sub population of thymocytes and splenocytes were modulated by the administration of PFVM[25]. In addition, water-soluble polysaccharide from *F. velutipes* (FVPI-A) resulted in increased Nitric Oxide (NO) and TNF-a production. The IL-1b and IL-6 was also increased and lymphocyte proliferation was promoted [21].

EFFECT OF *F. velutipes* STEM ON EXCRETA COMPOSITION AND GAS PRODUCTION

Higher excreta Dry Matter (DM) content can prevent the problems caused by wet litter in layer farms as well as increased the absorption of nutrients and reduce the ammonia gas production in faeces. The higher level of ammonia concentration in poultry houses is undesirable because ammonia is toxic and has been implicated for high chick's mortality and poor chicken growth[38]. In addition high concentrations of ammonia (NH_3) from manure impair productivity, including growth rate[39] and egg production[40] as well as damaging the respiratory tract[41]. Lee *et al.*[20] who observed that the addition of 4-5% fermented FV stem in the laying hens feed can decrease NH_3 gas emission in feces. *Flammulina velutipes* stem can be used in laying hen ration to improve the DM content of excreta which might help to reduce gas production as well as solve the problems caused by unexpected gas production in laying hen excreta.

CONCLUSION

Winter mushroom has been reported as immune enhancers, anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory and cholesterol lowering effect in many species including chicken. *Flammulina velutipes* stem has both nutritional and medicinal value for therapeutic use and pharmaceutical uses. The fruiting body and stem of *F. velutipes* mushroom have significant impact on improving chicken health and production performance. In fact, this review study observed that *F. velutipes* stem could improve the laying hen production performance like hen-day egg production and Feed Conversion Ratio (FCR). Egg quality parameters including egg weight, egg shell thickness, haugh unit, egg yolk cholesterol, yolk fatty acid were positively affected with *F. velutipes* stem supplementation in layer. Addition of winter mushroom stem improved gut health by altering small intestinal morphology and suppressing pathogenic bacteria in laying hen intestine. *Flammulina velutipes* stem also improved excreta DM and excreta NH_3 gas emission in layer. It could be suggested that *F. velutipes* stem may be used as a novel feed supplement in layer diet for effective utilization of natural resources as well as substitute to antibiotics. Finally, the above information represented in this review may help the basis knowledge about potential uses of *F. velutipes* stem in poultry ration for the future study.

SIGNIFICANCE STATEMENT

This study discover the *F. velutipes* mushroom stem as a potential feed supplementation as well as substitute of antibiotics that can be beneficial for laying performance, egg quality, intestinal morphology, immunity and gut health in layer chicken. This study will help the researcher to uncover the critical areas of immunity, gut health as well as egg quality that many researchers were not able to explore. Thus a new theory on improving the laying hen performance with *F. velutipes* mushroom stem may be arrived at.

REFERENCES

1. Tang, C., P.C.X. Hoo, L.T.H. Tan, P. Pusparajah and T.M. Khan *et al*., 2016. Golden needle mushroom: A culinary medicine with evidenced-based biological activities and health promoting properties. Front. Pharmacol., Vol. 7. 10.3389/fphar.2016.00474
2. Abe, F., N. Ishibashi and S. Shimamura, 1995. Effect of administration of Bifidobacteria and lactic acid bacteria to newborn calves and piglets. J. Dairy Sci., 78: 2838-2846.
3. Wasser, S.P. and A.L. Weis, 1999. Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: A modern perspective. Crit. Rev. Immunol., 19: 65-96.
4. Fard, S.H., M. Toghyani and S.A. Tabeidian, 2014. Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. Int. J. Recycl. Org. Waste Agric., 3: 141-146.
5. Yang, W., Y. Fang, J. Liang and Q. Hu, 2011. Optimization of ultrasonic extraction of *Flammulina velutipes* polysaccharides and evaluation of its acetylcholinesterase inhibitory activity. Food Res. Int., 44: 1269-1275.
6. Pang, X., W. Yao, X. Yang, C. Xie, D. Liu, J. Zhang and X. Gao, 2007. Purification, characterization and biological activity on hepatocytes of a polysaccharide from *Flammulina velutipes* mycelium. Carbohydr. Polym., 70: 291-297.
7. Wu, D.M., W.Q. Duan, Y. Liu and Y. Cen, 2010. Anti-inflammatory effect of the polysaccharides of golden needle mushroom in burned rats. Int. J. Biol. Macromol., 46: 100-103.
8. Smiderle, F.R., E.R. Carbonero, C.G. Mellinger, G.L. Sassaki, P.A.J. Gorin and M. Iacomini, 2006. Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*. Phytochemistry, 67: 2189-2196.
9. Hirai, Y., M. Ikeda, T. Murayama and T. Ohata, 1998. New monoterpentriols from the fruiting body of *Flammulina velutipes*. Biosci. Biotechnol. Biochem., 62: 1364-1368.

10. Wang, P.H., C.I. Hsu, S.C. Tang, Y.L. Huang, J.Y. Lin and J.L. Ko, 2004. Fungal immunomodulatory protein from *Flammulina velutipes* induces interferon-γ production through p38 mitogen activated protein kinase signaling pathway. J. Agric. Food Chem., 52: 2721-2725.
11. Wang, H., T.B. Ng and V.E.C. Ooi, 1998. Lectins from mushrooms. Mycol. Res., 102: 897-906.
12. Ishikawa, N.K., Y. Fukushi, K. Yamaji, S. Tahara and K. Takahashi, 2001. Antimicrobial cuparene-type sesquiterpenes, enokipodins C and D, from a mycelial culture of *Flammulina velutipes*. J. Nat. Prod., 64: 932-934.
13. Ishikawa, N.K., K. Yamaji, S. Tahara, Y. Fukushi and K. Takahashi, 2000. Highly oxidized cuparene-type sesquiterpenes from a mycelial culture of *Flammulina velutipes*. Phytochemistry, 54: 777-782.
14. Yi, C., C. Sun, S. Tong, X. Cao and Y. Feng *et al*., 2013. Cytotoxic effect of novel *Flammulina velutipes* sterols and its oral bioavailability via mixed micellar nanoformulation. Int. J. Pharm., 448: 44-50.
15. Ko, W.C., W.C. Liu, Y.T. Tsang and C.W. Hsieh, 2007. Kinetics of winter mushrooms (*Flammulina velutipes*) microstructure and quality changes during thermal processing. J. Food Eng., 81: 587-598.
16. Shen, J.W., S.M. Jia, J.J. Wu and J.H. Jia, 1997. Research on the nutrition composition of *Flammulina velutipes*. Edible Fungi China, 16: 36-39.
17. Na, J.C., B.G. Jang, S.H. Kim, J.H. Kim and S.K. Kim *et al*., 2005. Influence of feeding *Flammulina velutipes* media on productivity and egg quality in laying hens. Korean J. Poult. Sci., 32: 143-147.
18. Lee, S.B., Y.H. Choi, S.K. Cho, T.S. Shin and B.W. Cho *et al*., 2012. Effects of dietary *Flammulina velutipes* mycelium on broiler chick performance, pathogenic bacterial counts in caecal contents and amount of NH_3 in excreta. J. Anim. Sci. Technol., 54: 341-347.
19. Kim, M.Y., I.M. Chung, S.J. Lee, J.K. Ahn and E.H. Kim *et al*., 2009. Comparison of free amino acid, carbohydrates concentrations in Korean edible and medicinal mushrooms. Food Chem., 113: 386-393.
20. Lee, S.B., J. Im, S.K. Kim, Y.C. Kim, M.J. Kim, J.S. Lee and H.G. Lee, 2014. Effects of dietary fermented *Flammulina velutipes* mycelium on performance and egg quality in laying hens. Int. J. Poult. Sci., 13: 637-644.
21. Wu, M., X. Luo, X. Xu, W. Wei and M. Yu *et al*., 2014. Antioxidant and immunomodulatory activities of a polysaccharide from *Flammulina velutipes*. J. Tradit. Chin. Med., 34: 733-740.
22. Badalyan, S.M. and L.A. Hambardzumyan, 2001. Investigation of immunomodulating activity of the medicinal mushroom *Flammulina velutipes* (Curt.: Fr.) P. Karst. *in vitro*. Cytokine induction by fruiting body extract. Int. J. Med. Mushrooms, 3: 110-111.
23. Hwang, J.A., M.E. Hossain, D.H. Yun, S.T. Moon, G.M. Kim and C.J. Yang, 2012. Effect of shiitake [*Lentinula edodes* (Berk.) Pegler] mushroom on laying performance, egg quality, fatty acid composition and cholesterol concentration of eggs in layer chickens. J. Med. Plants Res., 6: 146-153.
24. Sun, Q., J. Ma, H. Campos, S.E. Hankinson and J.E. Manson *et al*., 2007. A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. Circulation, 115: 1858-1865.
25. Yeh, M.Y., W.C. Ko and L.Y. Lin, 2014. Hypolipidemic and antioxidant activity of enoki mushrooms (*Flammulina velutipes*). BioMed Res. Int. 10.1155/2014/352385
26. Ma, Z., C. Zhang, X. Gao, F. Cui and J. Zhang *et al*., 2015. Enzymatic and acidic degradation effect on intracellular polysaccharide of *Flammulina velutipes* SF-08. Int. J. Biol. Macromol., 73: 236-244.
27. Puttaraju, N.G., S.U. Venkateshaiah, S.M. Dharmesh, S.M.N. Urs and R. Somasundaram, 2006. Antioxidant activity of indigenous edible mushrooms. J. Agric. Food Chem., 54: 9764-9772.
28. Kim, M.Y., P. Seguin, J.K. Ahn, J.J. Kim and S.C. Chun *et al*., 2008. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem., 56: 7265-7270.
29. Tie, M., Y.Z. Fang, T.B. Sun, C. Li, J.Y. Fei, H.W. Li and S.L. Zang, 2007. Application of HPLC-ICP-MS in speciation analysis of selenium in selenized *Flammulina velutipes*. Chem. J. Chin. Univ., 28: 635-639.
30. James, M.J., V.M. Ursin and L.G. Cleland, 2003. Metabolism of stearidonic acid in human subjects: Comparison with the metabolism of other n-3 fatty acids. Am. J. Clin. Nutr., 77: 1140-1145.
31. Yashodhara, B.M., S. Umakanth, J.M. Pappachan, S.K. Bhat, R. Kamath and B.H. Choo, 2009. Omega-3 fatty acids: A comprehensive review of their role in health and disease. Postgraduate Med. J., 85: 84-90.
32. Ergonul, P.G., I. Akata, F. Kalyoncu and B. Ergonul, 2013. Fatty acid compositions of six wild edible mushroom species. Scient. World J. 10.1155/2013/163964
33. Giannenas, I., D. Tontis, E. Tsalie, E. Chronis, D. Doukas and I. Kyriazakis, 2010. Influence of dietary mushroom *Agaricus bisporus* on intestinal morphology and microflora composition in broiler chickens. Res. Vet. Sci., 89: 78-84.
34. Zeng, Q.L., H. Song, H.H. Guo, C. Guo, D. Zhen, F. Liang and T. Zhang, 2016. Effect of supplementation of *Flammulina velutipes* stem base on the caecal microflora, short chain fatty acid of broiler. J. China Agric. Univ., 21: 104-114.
35. Leung, M.Y.K., K.P. Fung and Y.M. Choy, 1997. The isolation and characterization of an immunomodulatory and anti-tumor polysaccharide preparation from *Flammulina velutipes*. Immunopharmacology, 35: 255-263.

36. Guo, F.C., B.A. Williams, R.P. Kwakkel, H.S. Li and X.P. Li *et al*., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci., 83: 175-182.
37. Willis, W.L., O.S. Isikhuemhen and S.A. Ibrahim, 2007. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult. Sci., 86: 1856-1860.
38. Maliselo, P.S. and G.K. Nkonde, 2015. Ammonia production in poultry houses and its effect on the growth of *Gallus gallus domestica* (broiler chickens): A case study of a small scale poultry house in Riverside, Kitwe, Zambia. Int. J. Scient. Technol. Res., 4: 141-145.
39. Reece, F.N., B.J. Bates and B.D. Lott, 1979. Ammonia control in broiler houses. Poult. Sci., 58: 754-760.
40. Deaton, J.W., F.N. Reece and B.D. Lott, 1984. Effect of atmospheric ammonia on pullets at point of lay. Poult. Sci., 63: 384-385.
41. Nagaraja, K.V., D.A. Emercy, K.A. Jordan, J.A. Newman and B.S. Pomeroy, 1983. Scanning electron microscopic studies of adverse effects of ammonia on tracheal tissues of turkeys. Am. J. Vet. Res., 44: 1530-1536.