PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

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

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2017.696.699

Research Article
Effect of Dietary Supplementation of Chitosan on Blood Biochemical Profile of Laying Hens

[1]Elvia Hernawan, [1]Lovita Adriani, [1]Andi Mushawwir, [2]Chandrawati Cahyani and [3]Darmawan

[1]Department of Animal Husbandry, University Padjadjaran, West Java, Indonesia
[2]Department of Chemical Engineering, Brawijaya University, Malang East Java, Indonesia
[3]National Nuclear Energy Agency, Indonesia

Abstract

Background and Objective: Chitosan is a natural alkaline polysaccharide and widespread in nature. A study was conducted to evaluate the effect of dietary supplementation of chitosan on blood biochemical parameters like cholesterol, malondialdehyde (MDA), creatinine and total leucocytes. **Methodology:** One hundred laying phase hens, aged 28 weeks were used in the study. The birds were divided into 2 treatment groups each having 3 replicates of 10 birds each. Group I served as control and was fed basal diet. In Group II, basal diet was supplemented with chitosan at 150 ppm g^{-1} by spraying method. **Results:** The results showed that the chitosan inclusion in the diet of layer pullets significantly ($p<0.05$) lowered the total cholesterol (36.749 ± 0.381 mg dL^{-1}) when compared to the control group (43.030 ± 0.352 mg dL^{-1}). The dietary incorporation of chitosan significantly ($p<0.05$) decreased blood MDA levels (1.829 ± 0.237 nmoles mL^{-1}) of laying hens compared to control (2.553 ± 0.379 nmoles mL^{-1}). The creatinine levels also decreased significantly ($p<0.05$) in birds fed diet supplemented with chitosan, however, there was no effect on the total leucocyte count. **Conclusion:** The incorporation of chitosan in the diet had positive effect in terms of reducing the blood cholesterol and malondialdehyde levels of laying hens.

Key words: Chitosan, cholesterol, creatinine, laying hens, malondialdehyde

Received: December 13, 2016 Accepted: June 21, 2017 Published: August 15, 2017

Citation: Elvia Hernawan, Lovita Adriani, Andi Mushawwir, Chandrawati Cahyani and Darmawan, 2017. Effect of dietary supplementation of chitosan on blood biochemical profile of laying hens. Pak. J. Nutr., 16: 696-699.

Corresponding Author: Lovita Adriani, Department of Animal Husbandry, University Padjadjaran, West Java, Indonesia

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

Chitosan, a deacetylated chitin, is widespread in nature. The exoskeletons of arthropods such as crabs, shrimps, insects and other marine creatures in the *Crustacean* family are good sources of chitosan[1-3]. Chitosan is a natural alkaline polysaccharide with positive charge and also one of the most abundant natural polymers[4-6]. Unlike chitin, chitosan oligosaccharide (COS) is soluble in acidic solutions[7] and it is partially digested in the gastrointestinal tract of monogastric animals[8,9]. Chitosan is commercially manufactured from chitin, in the process of deacetylation, by treating chitin with a strong solution of sodium hydroxide at an elevated temperature[10]. As chitosan and its oligosaccharide derivatives contain reactive, functional groups, that is, amino acids and hydroxyl groups, they have unlike chitin, antimicrobial[11,12], anti-inflammatory[13,14], anti-oxidative[15,16], immunostimulatory[17,18] and hypocholesterolemic[19,20] properties.

Cholesterol level in meat has great importance as hypercholesterolemia is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarctions, which is a common cause of mortality and morbidity in humans[21,22]. Many people limit meat consumption because of fear of elevated serum cholesterol concentrations[23]. Malondialdehyde (MDA) is the direct product of lipid peroxidation developed after radical attack and thus is an indicator of the extent of cell damage[24]. The MDA has been reported to cause fragmentation of destruction of cell membrane structure, deoxyribonucleic acid (DNA) and accelerate apoptosis[25], thus warrants estimation of its levels as well. Creatinine is an important indicator of protein metabolism and its concentration is directly proportional to muscle mass, age, physical activity and diet[26].

In view of the above, a study was conducted to evaluate the effect of dietary supplementation of chitosan on blood biochemical parameters like cholesterol, malondialdehyde (MDA), creatinine and total leucocytes.

MATERIALS AND METHODS

Birds and management: The experiment was carried out at CV Acum, Kuningan West Java, Indonesia. The study was conducted on 100 laying phase hens (having average body weight of 1452±23 g) aged 28 weeks. The birds were divided into 2 treatment groups each having 3 replicates of 10 birds each. Group I served as control and was fed basal diet. The composition of the basal diet was as follows: Total protein 18%, fat 8%, crude fiber 4%, methionine 0.4%, cysteine 0.3%, tryptophan 0.18%, lysine 0.9%, calcium 1% and phosphorus 0.5%. In Group II, basal diet was supplemented with chitosan at 150 ppm g^{-1} by spraying method.

Processing of chitosan: Low molecular weight chitosan with average molecular weight (Mw) of 7×10^3 Da (oligochitosan or irradiated chitosan) was provided by Center for Isotopes and Radiation Application (CIRA), National Nuclear Energy Agency (NNEA) Indonesia. The chitosan was prepared by irradiating the high molecular weight (Mw) chitosan of (1.5×10^5 Da) using gamma rays from Cobalt-60 source according to procedures in PATENT No IDP000034713 (15a).

Parameters recorded: Blood was collected after the end of trail (6 weeks) from all the birds. The parameters recorded were cholesterol, MDA, creatinine and total leucocytes. The cholesterol was estimated by the help of cholesterol kit using method of CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol) as per Richmond[27]. The MDA was estimated using a modified test method thiobarbituric acid (TBA) by spectrophotometry as per Zainuri and Wanandi[28]. The collected blood sample 400 mL was treated with 200 mL of 20% trichloroacetic acid (TCA). The vortex was then applied to the resultant mixture and afterwards centrifuged at 5000 rpm for 10 min. The supernatant formed was collected and 400 mL of 0.67% TBA was added to it. The sample was again mixed thoroughly with vortex and incubated in water bath at a temperature of 96°C for 10 min. The mixture was then removed and allowed to cool at room temperature. The absorbance was then read at a wavelength of 530 nm and MDA level was expressed as nmoles mL^{-1}. The creatinine was estimated as per the method of Underwood[29]. Total leucocytes were determined by means of hematological analyzer (KT-6200 VET) and expressed as 10^3 mm^{-3}.

Statistical analysis: Data collected were subjected to one way analysis of variance (ANOVA) as per Steel and Torrie[30] and Duncan's multiple range test[31] was used to test the significance of difference between means considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The results showed that the chitosan inclusion in the diet of layer pullets significantly ($p<0.05$) lowered the total cholesterol when compared to the control group. Several factors such as a diet rich in cholesterol has been reported to cause heart failure[32]. Further, the high levels of cholesterol, particularly low-density lipoprotein (LDL), are mainly

Table 1: Blood biochemical profile of laying hens fed chitosan in the diet

Treatments	Values
Cholesterol (mg dL^{-1})	
Group I (Control)	43.030±0.352^{a}
Group II	36.749±0.381^{b}
MDA (nmoles mL^{-1})	
Group I (Control)	2.553±0.379^{a}
Group II	1.829±0.237^{b}
Creatinine (mg dL^{-1})	
Group I (Control)	6.004±0.003^{a}
Group II	5.042±0.021^{b}
Total leucocytes (×10^{3} mm^{-3})	
Group I (Control)	86.20±0.033^{a}
Group II	82.34±0.022^{a}

Means within the rows with different superscripts are significantly different (p>0.05)

responsible for hypercholesterolemia[22], which is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infection[21]. Therefore, inclusion of chitosan in the diet of poultry birds could be an important asset for health conscious people, however, the mechanism by which chitosan reduces blood cholesterol in poultry birds is not clear.

The dietary incorporation of cholesterol significantly (p<0.05) decreased blood MDA levels of laying hens. Malondialdehyde (MDA) is the direct product of lipid peroxidation developed after radical attack and thus is an indicator of the extent of cell damage[24]. The MDA has been reported to cause fragmentation of destruction of cell membrane structure, deoxyribonucleic acid (DNA) and accelerate apoptosis[25]. The results of present study thus indicated that chitosan helped the birds in alleviating day to day stress as indicated by the decreased MDA levels in chitosan fed group.

The creatinine levels also decreased significantly (p<0.05) in birds fed diet supplemented with chitosan. High creatinine levels are indicative of increased protein metabolism or kidney problems. Therefore, it is suggested that the chitosan incorporation in the diet of laying hens has no adverse effect on the kidneys of birds. Moreover, there was no effect on the total leucocyte count as a result of dietary incorporation of chitosan in layer birds, indicating that there was not any pathological condition associated with the inclusion of chitosan in the diet (Table 1).

CONCLUSION

The incorporation of chitosan in the diet of laying hens had positive effect in terms of reducing the blood cholesterol and malondialdehyde levels without having any pathological effect on the birds. However, further studies are needed to evaluate the mechanism by which chitosan helps in achieving such positive effects.

ACKNOWLEDGMENT

The authors would like to express special gratitude to the students in chitosan group as well the principal who helped a lot during the present study.

REFERENCES

1. Crini, G., 2005. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Prog. Polym. Sci., 30: 38-70.
2. Huang, R.L., Y.L. Yin, G.Y. Wu, Y.G. Zhang and T.J. Li *et al.*, 2005. Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poult. Sci., 84: 1383-1388.
3. Li, H.Y., S.M. Yan, B.L. Shi and X.Y. Guo, 2009. Effect of chitosan on nitric oxide content and inducible nitric oxide synthase activity in serum and expression of inducible nitric oxide synthase mRNA in small intestine of broiler chickens. Asian-Aust. J. Anim. Sci., 22: 1048-1053.
4. Knaul, J.Z., S.M. Hudson and K.A.M. Creber, 1999. Crosslinking of chitosan fibers with dialdehydes: Proposal of a new reaction mechanism. J. Polym. Sci. Part B: Polym. Phys., 37: 1079-1094.
5. Wang, S.Q. and C.S. Zhang, 2004. Chitin, chitosan and their application in aquaculture. Feed Res., 5: 25-28.
6. Xu, C.L. and Y.Z. Wang, 2005. Application of chitin in aquiculture. China Feed, 7: 30-32.
7. Shahidi, F., J.K.V. Arachchi and Y.J. Jeon, 1999. Food applications of chitin and chitosans-review. Trends Food Sci. Technol., 10: 37-51.
8. Hirano, S., C. Itakura, H. Seino, Y. Akiyama, I. Nonata, N. Kanbara and T. Kawahami, 1990. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem., 38: 1214-1217.
9. Okamoto, Y., M. Nose, K. Miyatake, J. Sekine, R. Oura, Y. Shigemasa and S. Minami, 2001. Physical changes of chitin and chitosan in canine gastrointestinal tract. Carbohydr. Polym., 44: 211-215.
10. Singla, A.K. and M. Chawla, 2001. Chitosan: Some pharmaceutical and biological aspects-an update. J. Pharm. Pharmacol., 53: 1047-1067.
11. Zheng, L.Y. and J.F. Zhu, 2003. Study on antimicrobial activity of chitosan with different molecular weights. Carbohydr. Polym., 54: 527-530.
12. Holappa, J., M. Hjalmarsdottir, M. Masson, O. Runarsson and T. Asplund *et al.*, 2006. Antimicrobial activity of chitosan N-betainates. Carbohydr. Polym., 65: 114-118.
13. Yoon, H.J., M.E. Moon, H.S. Park, S.Y. Im and Y.H. Kim, 2007. Chitosan Oligosaccharide (COS) inhibits LPS-induced inflammatory effects in RAW 264.7 macrophage cells. Biochem. Biophys. Res. Commun., 358: 954-959.

14. Ma, P., H.T. Liu, P. Wei, Q.S. Xu, X.F. Bai, Y.G. Du and C. Yu, 2011. Chitosan oligosaccharides inhibit LPS-induced over-expression of IL-6 and TNF-α in RAW264.7 macrophage cells through blockade of Mitogen-Activated Protein Kinase (MAPK) and PI3K/Akt signaling pathways. Carbohydr. Polym., 84: 1391-1398.
15. Kim, K.W. and R.L. Thomas, 2007. Antioxidative activity of chitosans with varying molecular weights. Food Chem., 101: 308-313.
16. Yen, M.T., J.H. Yang and J.L. Mau, 2008. Antioxidant properties of chitosan from crab shells. Carbohydr. Polym., 74: 840-844.
17. Nishimura, K., S.I. Nishimura, N. Nishi, F. Numata, Y. Tone, S. Tokura and I. Azuma, 1985. Adjuvant activity of chitin derivatives in mice and guinea-pigs. Vaccine, 3: 379-384.
18. Zaharoff, D.A., C.J. Rogers, K.W. Hance, J. Schlom and J.W. Greiner, 2007. Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. Vaccine, 25: 2085-2094.
19. Ormrod, D.J., C.C. Holmes and T.E. Miller, 1998. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis, 138: 329-334.
20. Liu, J., J. Zhang and W.S. Xia, 2008. Hypocholesterolaemic effects of different chitosan samples *in vitro* and *in vivo*. Food Chem., 107: 419-425.
21. Wald, N.J. and M.R. Law, 1995. Serum cholesterol and ischaemic heart disease. Atherosclerosis, 118: S1-S5.
22. Krieger, M., 1998. The best of cholesterols, the worst of cholesterols: A tale of two receptors. Proc. Natl. Acad. Sci. USA., 95: 4077-4080.
23. Abeywardena, M.Y., 2003. Dietary fats, carbohydrates and vascular disease: Sri Lankan perspectives. Atherosclerosis, 171: 157-161.
24. Puvaca, N., L. Kostadinovic, S. Popovic, J. Levic and D. Ljubojevic *et al*., 2016. Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum*, *Piper nigrum*, *Capsicum annuum*). Anim. Prod. Sci., 56: 1920-1927.
25. Wang, H.F., X.H. Zhong, W.Y. Shi and B. Guo, 2011. Study of malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus. Afr. J. Biotechnol., 10: 9213-9217.
26. Szabo, A., M. Mezes, P. Horn, Z. Suto, G.Y. Bazar and R. Romvari, 2005. Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). Acta Vet. Hung., 53: 397-409.
27. Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.
28. Zainuri, M. and S.I. Wanandi, 2012. [Spesific activity of Manganese Superoxide Dismutase (MnSOD) and catalase in the rat liver induced systemic hypoxia: Relationship with oxidative damage]. Media Litbang Kesehatan, 22: 87-92, (In Indonesian).
29. Underwood, J.C.E., 1997. General and Systematic Pathology. Publishers Books Medicine EGC., Jakarta, Indonesia.
30. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometral Approach. McGraw-Hill Book Company, New York.
31. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
32. Schaefer, E.J., A.H. Lichtenstein, S. Lamon-Fava, J.R. McNamara and J.M. Ordovas, 1995. Lipoproteins, nutrition, aging and atherosclerosis. Am. J. Clin. Nutr., 61: 726S-740S.