Dietary encapsulated probiotic effect on broiler serum biochemical parameters

P. Yazhini¹, P. Visha², P. Selvaraj³, P. Vasanthakumar³ and V. Chandran¹

1. Suguna Institute of Poultry Management, Udumalpet, Tirupur, Tamil Nadu, India; 2. Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India; 3. Faculty Veterinary University Training and Research Centre, Karur, Tamil Nadu, India.

Corresponding author: P. Yazhini, e-mail: palaniyazhini@gmail.com
Co-authors: PV: visha.p@tanuvas.ac.in, PS: p.selvaraj@tanuvas.ac.in, PV: vasanthakumar.p@tanuvas.ac.in, VC: drchandranvet07@gmail.com

Received: 07-04-2018, Accepted: 10-08-2018, Published online: 29-09-2018

doi: 10.14202/vetworld.2018.1344-1348 How to cite this article: Yazhini P, Visha P, Selvaraj P, Vasanthakumar P, Chandran V (2018) Dietary encapsulated probiotic effect on broiler serum biochemical parameters, Veterinary World, 11(9): 1344-1348.

Abstract

Aim: The study aimed to evaluate the effect of encapsulated probiotic bacteria (Lactobacillus lactis and Bifidobacterium bifidum) on broiler serum biochemical parameters.

Materials and Methods: Encapsulation protects the probiotics and increases their livability on exposure to unfavorable processing and storage temperatures and gastrointestinal pH. Hence, an in vitro study was undertaken to encapsulate the probiotic bacteria L. lactis and B. bifidum with sodium alginate and chitosan and evaluate the encapsulation efficiency. This experiment was conducted with 288-day-old broiler chicken; they were distributed randomly into eight treatments and six replicates in each treatment (six birds in each replicate) and given with standard feed.

Results: Supplementation of the encapsulated bacteria either alone or in combination (T1, T2, and T3) significantly (p<0.05) increased mean total serum protein, albumin, and globulin as compared to the birds that were not supplemented with any probiotic (T4 and T5) or supplemented with non-encapsulated bacteria (T6, T7, and T8). Supplementation of the encapsulated bacteria either alone or in combination (T1, T2, and T3) significantly (p<0.05) lowered mean total serum cholesterol, serum low-density lipoprotein (LDL) cholesterol, and serum triglycerides, as compared to the birds that were not supplemented with any probiotic (T4 and T5) or supplemented with non-encapsulated bacteria (T6, T7, and T8).

Conclusion: It may be concluded that supplementation of the encapsulated probiotic bacteria either alone or in combination significantly increased total serum protein, albumin, and globulin and significantly lowered mean total serum cholesterol, serum LDL cholesterol, and serum triglycerides as compared to the birds that were not supplemented with any probiotic or supplemented with non-encapsulated bacteria.

Keywords: biochemical, broiler, encapsulated, probiotic, serum.

Introduction

Along with the increase in demand for quality of animal product, concerns about the effects of these products on human health are also increasing. Hence, the focus should be made not only on high productivity but also on their impact on human health and the environment. Even though antibiotics are shown to increase production in broiler industries, focus on antibiotic resistant also increased. To provide good quality broiler meat without compromising, the production level probiotics are shown to be the best way. Probiotics can be defined as live microorganisms which, when administrated in adequate numbers, confer health benefits to the host by improving the microbial balance [1,2]. Many research studies have reported that inclusion of probiotic species such as Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces in broiler nutrition has a beneficial effect on growth performance, intestinal health, immune status, and meat characteristics such as microbial load, keeping quality, and sensory evaluation [3].

While supplementing probiotics to birds and animals, activity and stability of probiotic microorganism are affected by different storage temperature, stability in dried and frozen form, acidic and alkaline pH of gastrointestinal tract, [4]. Many reports have indicated that there is poor survival of bacteria in products containing free probiotic cells during passage through the upper gastrointestinal system [5].

Encapsulation is the process which enhances the survivability and stability of probiotic bacteria. There are various methods employed in encapsulation among them encapsulation of probiotic bacteria with alginate and chitosan provides protection in simulated gastrointestinal condition, and therefore, it is a good way of delivering viable bacteria cells to the intestine [6].

The study aimed to encapsulate the probiotic bacteria (Lactobacillus lactis and Bifidobacterium bifidum) for supplementation through feed and to evaluate the
effect of encapsulated probiotic bacteria on broiler serum biochemical index.

Materials and Methods

Ethical approval

This study was approved by Tamil Nadu Veterinary and Animal Sciences University Ethical Committee.

Encapsulation

Encapsulation of Lactobacillus lactis and Bifidobacterium bifidum was carried out separately following the method of Sharma et al. [7] with slight modifications. 100 mg of probiotic culture (L. lactis/B. bifidum) was inoculated in 5 mL of specific broth DeMan Rogosa and Sharpe (MRS) broth for L. lactis and Bifidobacterium broth for B. bifidum and incubated anaerobically at 37°C for overnight. After incubation, the culture was centrifuged at 4000 rpm for 30 min, and the supernatant was removed. The bacterial pellet was washed thrice with phosphate-buffered saline (PBS) (pH - 7.0), resuspended in 1 mL PBS, and then mixed with 10 mL of 4% autoclaved sodium alginate solution. This mixture was extruded using a sterile insulin syringe into 100 mL of gently stirred autoclaved 2.5% calcium chloride solution with the help of a magnetic stirrer. The distance between the tip of the syringe and CaCl2 solution was 30 cm. The droplets formed gel spheres instantaneously. The beads were left in the hardening solution for 30 min and then transferred into 100 mL of the coating solution (autoclaved 0.4% chitosan solution) and left for 30 min with constant stirring. The dried beads were stored in sterile vials until further use (biological trial).

Experimental birds and diet

A total of 288 numbers of day-old broiler chicks were wing banded, weighed, and randomly allotted to eight groups with six replicates of six chicks each based on the body weight with all replicates having similar body weight. The dietary supplementation of non-encapsulated and encapsulated bacteria (L. lactis and B. bifidum) followed as mentioned below.

The treatment groups consisted of basal diet T1 (basal diet with antibiotic), T2 (basal diet without antibiotic), T3 (basal diet + non-encapsulated L. lactis 1×106 cfu/kg feed), T4 (basal diet + encapsulated L. lactis 1×106 cfu/kg feed), T5 (basal diet + non-encapsulated B. bifidum 1×1012 cfu/kg feed), T6 (basal diet + encapsulated B. bifidum 1×1012 cfu/kg feed), T7 (basal diet + non-encapsulated L. lactis 1×106 cfu/kg feed and B. bifidum 1×106 cfu/kg feed), and T8 (basal diet + encapsulated L. lactis 1×106 cfu/kg feed and B. bifidum 1×106 cfu/kg feed). The probiotic-supplemented groups were fed basal diets without any antibiotic.

Blood collection

At the end of 4th, 5th, and 6th week of age, 5 mL blood samples were collected using 23G sterile needles from wing vein of six birds per treatment. Plasma samples for the estimation of biochemical constituents were obtained by collecting blood in sterile tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Serum samples for hemagglutination titer assessment were obtained by collecting blood in sterile tubes without EDTA.

Estimation of blood parameters

The blood biochemicals were analyzed using commercial kits (Span Diagnostics Ltd., India) in UV-visible double beam spectrophotometer (SYSTRONICS, Model 2202, India). Total cholesterol content, high-density lipoprotein (HDL) cholesterol [8], glucose [9], triglycerides [10], total protein [11], albumin, and globulin [9] content were estimated in the plasma.

Statistical analysis

The data collected on various parameters were grouped and subjected to statistical analysis by one-way ANOVA using SPSS, version 20.0 for Windows (IBM, USA).

Results

Effect on total proteins, albumin, and globulin

The effect of supplementation of non-encapsulated and encapsulated probiotic bacteria alone and their combination on the mean total proteins, albumin, and globulin (g/dL) in broiler chicken is presented in Table-1.

The mean plasma total protein values of T1 and T2 were similar and significantly (p<0.05) lower as compared to all other treatment groups. The mean plasma total protein values between T5, T6, and T7 were similar and significantly (p<0.05) higher than all the other treatment groups. There was no significant difference in mean protein values among T3, T4, and T8 groups.

The mean plasma albumin value was significantly (p<0.05) lowest in T1 and T2 groups as compared to all the other treatment groups. T8 group of birds showed significantly (p<0.05) higher mean plasma albumin values. There was no significant (p>0.05) difference among plasma albumin values of birds belonging to T3, T4, and T6 and between T5 and T7.

The mean plasma globulin value was significantly (p<0.05) lowest in T1 and T2 groups of broiler chicken in comparison to all the other treatment’s groups. There was no significant (p>0.05) difference in the plasma albumin values of birds belonging to T3, T4, T5, T6, T7, and T8 groups of birds showed significantly (p>0.05) higher mean plasma globulin values as compared to the other treatment groups.

Effect on plasma lipid profile

The effect of supplementation of non-encapsulated and encapsulated probiotic bacteria alone and their combination on the mean lipid profile in the broiler chicken is presented in Table-2.

Plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, and HDL cholesterol

The mean plasma total cholesterol levels were significantly (p<0.05) highest in T4 and T5 groups of broilers when compared with all other treatment
groups. Among probiotic-supplemented groups, the mean plasma cholesterol level was significantly (p<0.05) lower in the T8 group. In general, encapsulated probiotic-supplemented group had significantly (p<0.05) lower mean plasma total cholesterol as compared to the birds supplemented with non-encapsulated probiotic-supplemented groups.

The mean plasma LDL cholesterol level was significantly (p<0.05) higher in the T1 and T2 groups in comparison to all the other treatment groups. There was no significant (p>0.05) difference in the mean plasma LDL cholesterol level among the treatment groups T4 and T6 and between T4, T5, and T6. The mean plasma LDL cholesterol level was significantly (p<0.05) lower in T8 group of broiler chickens in comparison to all other treatment groups.

The mean plasma HDL cholesterol level was significantly (p<0.05) lower in the T1 and T2 group of broiler chicken in comparison to all the other treatments and controls. The lowest plasma triglycerides level was recorded in T8 group. The groups of birds which received the encapsulated bacteria (T4, T5, and T6) had significantly (p<0.05) lower mean total triglycerides as compared to the birds that supplemented with non-encapsulated bacteria (T1, T2, and T3).

Discussion

Effect on total proteins, albumin, and globulin

The result of the present study concurs with that of Siadati et al. [12] who observed that probiotic supplementation increased the plasma protein and improved the growth performance in Japanese Quail. However, the results of the present study did not agree with findings of Li et al. [13] (Lactobacillus sporogenes) and Abdel-Hafeez et al. [14] who observed that the serum total protein concentration of birds supplemented with probiotic was significantly (p<0.05) lower than the control birds.

The lactic acid bacteria competitively exclude the pathogenic bacteria which reduce the breakdown of proteins to nitrogen and reduce the efficiency of dietary protein [15]. Thus, the utilization of amino acids and proteins is improved. Furthermore, increased villi height in the encapsulated probiotic-supplemented group as evident in the present study could have increased the protein absorption.

Table-2: Mean (± SE) lipid-protein profile (mg/dl) of broiler chicken supplemented with non-encapsulated and encapsulated probiotic bacteria.

| Treatment groups | Total cholesterol | HDL cholesterol | LDL cholesterol | Triglycerides |
|------------------|-------------------|-----------------|-----------------|--------------|
| Tb - basal diet+50 mg oxytetracycline/kg | 198.7±1.1 | 67.7±0.2 | 111.2±1.3 | 103±0.3 |
| Tb - basal diet+ (without antibiotic) | 199.5±2.3 | 66.6±1.1 | 112.1±2.1 | 104±0.1 |
| Tb-T1+non-encapsulated Lactobacillus lactis | 184±0.2 | 70.8±0.3 | 93.4±1.2 | 98.8±0.1 |
| Tb-T1+encapsulated Lactobacillus lactis | 179.3±0.1 | 77.7±0.2 | 84.9±0.2 | 87.6±0.1 |
| Tb-T2+non-encapsulated Bifidobacterium bifidum | 186.8±0.4 | 71.2±0.2 | 95.8±2.7 | 96.8±0.1 |
| Tb-T2+encapsulated Bifidobacterium bifidum | 179.6±0.2 | 77.6±0.3 | 82.4±0.4 | 87.6±0.1 |
| Tb-T2+non-encapsulated Lactobacillus lactis+ Bifidobacterium bifidum | 181±0.1 | 76.4±1.0 | 85.5±0.8 | 95.1±0.3 |
| Tb-T2+encapsulated Lactobacillus lactis+ Bifidobacterium bifidum | 171.4±2.5 | 80.6±0.1 | 73.7±2.4 | 85.5±0.1 |

Means within the same column bearing different superscripts differ significantly (p<0.05), HDL=High-density lipoprotein, LDL=Low-density lipoprotein.
In the present study, the groups of birds which received the encapsulated bacteria either alone or in combination \((T_s, T_o, T_e, \text{and } T_r)\) had significantly (\(p<0.05\)) higher mean serum total protein, albumin, and globulin values compared to the birds that were not supplemented with any probiotic \((T_s, T_o, \text{and } T_e)\) or supplemented with non-encapsulated bacteria \((T_{s}, T_{o}, \text{and } T_{e})\).

**Effect on plasma total cholesterol, LDL cholesterol, and HDL cholesterol**

The results of the present study concur with the findings of Siadati et al. [12], Ashayerizadeh et al. [16], and Iqramu et al. [17], who reported decreased serum total cholesterol level on supplementation of probiotic in poultry. However, the results of the present study did not agree with the findings of Shirisha et al. [18], who observed no significant difference in total cholesterol level between probiotic-supplemented birds and control birds.

The hypcholesterolemic effect observed in all the probiotic-supplemented broiler chicken may be due to mediated by various mechanisms. Lactic acid bacteria reduces the cholesterol by assimilating endogenous or exogenous originated cholesterol in the intestinal tract [19], reduces or inhibits the expression levels of Niemann-Pick C1-like 1 a protein, expressed on the surface of enterocytes, which reduces the cholesterol absorption [20]. Lactic acid bacteria produce bile salt hydrolase, enzyme which is responsible for deconjugation of bile salts and it helps to excrete more bile acids in the feces [21].

In our study too, mean plasma HDL cholesterol level was significantly \((p<0.05)\) highest in the \(T_o\) and \(T_e\) group of broiler chicken in comparison to all the other treatments. There were no significant differences in the mean plasma HDL cholesterol level among the treatment groups \(T_{s}, T_{o}, \text{and } T_{e}\). The mean plasma HDL cholesterol level was significantly \((p<0.05)\) lowest in the \(T_s\) group of birds in comparison to all the other treatment groups.

These results concur with the findings of Kalavathy et al. [22] who reported that probiotic supplementation decreased serum LDL level and but had no significant effect on serum HDL level broiler chickens. However, these findings did not agree with Ashayerizadeh et al. [16] who reported that supplementation of probiotic did not affect serum HDL and LDL concentration.

Supplementation of probiotic bacteria to broiler chicken altered the lipoprotein metabolism of birds favorably with more pronounced reduction on the total cholesterol and LDL cholesterol and increased HDL cholesterol concentration.

**Effect on plasma triglycerides**

The results of the present study concur with the findings of Al-Saad et al. [23], Kalavathy et al. [22], Ashayerizadeh et al. [24], Abeer et al. [25], Iqramu et al. [17], and Ashayerizadeh et al. [16], who reported significant decrease in serum triglycerides level in broiler chickens on probiotic supplementation.

However, the results of the present study differ with the findings of Haddadin et al. [26], who reported no significant reduction in triglyceride level in serum and eggs on supplementation of *Lactobacillus acidophilus* in laying hens.

**Conclusion**

Encapsulated bacteria either alone or in combination significantly increased total serum protein, albumin, and globulin values. Supplementation of the encapsulated bacteria either alone or in combination significantly lowered mean total cholesterol, LDL cholesterol, and triglycerides in comparison to the birds that were not supplemented with any probiotic or supplemented with non-encapsulated bacteria.

**Authors’ Contributions**

PY, PS, and PV contributed to the planning and doing research work as study design and writing. PVK contributed to the nutritional aspects of the research. VC contributed for writing. All authors read and approved the final manuscript.

**Acknowledgments**

The authors are thankful to the Tamil Nadu Veterinary and Animal Sciences University in Chennai for providing facilities and fund to complete this study. This study was conducted as a part of postgraduate research work.

**Competing Interests**

The authors declare that they have no competing interests.

**References**

1. Lee, S.H., Ingale, S.L., Kim, J.S., Kim, K.H., Anushka, L., Kim, E.K., Kwon, I.K., Kim, Y.H. and Chae, B.J. (2014) Effects of dietary supplementation with *Bacillus subtilis* LS-1-2 fermentation biomass on growth performance, nutrient digestibility, cecal microbiota and intestinal morphology of weaning pig. *Anim. Feed. Sci. Tech.*, 188: 102-110.
2. Zhang, Z.P. and Kim, I.H. (2014) Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poult. Sci.*, 93: 364-370.
3. Kabir, S.M.L. (2009) The role of probiotics in the poultry industry. *J. Mol. Sci.*, 10: 3531-3546.
4. Vinderola, C.G. and Reinheimer, J.A. (2000). Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*, bifidobacteria and lactic acid starter bacteria in fermented dairy products. *Int. Dairy J.*, 10: 271-275.
5. De Vos, P., Faas, M.M., Spasojevic, M. and Sikkema, J. (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *Int. Dairy J.*, 20: 292-302.
6. Chavarri, M., Maranon, I., Ares, R., Ibanez, F.C., Marzo, F. and Villaran, M.C. (2010) Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *Int. J. Food Microbiol.*, 142: 185-189.
7. Sharma, A., Bhatia, A., Singla, R. and Kaur, G. (2012) Improvement in bioactivity of *Lactobacillus* isolates by encapsulation in sodium alginate beads: *In vitro*. *Ann. Biol. Res.*, 3: 5403-5408.
8. Herbert, K. (1984) In: Kaplan, L.A. and Pesce, A.J.,
9. Tietz, N.W. (1976) Fundamentals of Clinical Chemistry. 2nd ed. W.B. Saunders Company Ltd., Philadelphia, PA. p246.

10. McGowan, M.W., Artiss, J.D., Strandbergh, D.R. and Zak, B. (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29: 538-542.

11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-269.

12. Siadati, S.A., Ebrahimnezhad, Y., Gh, S.J. and Shayeigh, J. (2017) Evaluation of probiotic potential of some native Lactobacillus strains on the growth performance and serum biochemical parameters of Japanese quails (Coturnix Coturnix japonica) during rearing period. Bras. J. Poult. Sci., 19: 399-408.

13. Li, Y., Xu, Q., Yang, C., Yang, X., Lv, L., Yin, C., Liu, X. and Yan, H. (2014) Effects of probiotics on the growth performance and intestinal microflora of broiler chickens. Pak. J. Pharm. Sci., 27: 713-717.

14. Abdel-Hafeez, H.M., Saleh, E.S.E., Tawfeek, S.S., Youssef, I.M.I. and Abdel-Daim, A.S.A. (2017) Effects of probiotic, prebiotic, and symbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian Aust. J. Anim. Sci., 30(5): 672-682.

15. Mikulec, Z., Serman, V., Mas, N. and Lukac, Z. (1999) Effect of probiotic on production results of fattened chickens fed different quantities of protein. Vet. Arhiv., 69: 199-209.

16. Ashayerizadeh, A., Dabiri, N., Mirzadeh, K.H. and Ghorbani, M.R. (2011) Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. J. Cell Anim. Biol., 5: 152-156.

17. Iqramu, M.H., Nazim, A. and Mohammad A.M. (2017) Comparative analysis of body weight and serum biochemistry in broilers supplemented with some selected probiotics and antibiotic growth promoters. J. Adv. Vet. Anim. Res., 4: 288-294.

18. Shirisha, R., Krishnadada, Raju, M.V.L.N., Sai, R.S. and Ravinder, R.V. (2017) Effect of dietary supplementation of probiotic (problend) on Immune Status, biochemical profile and E. coli counts in commercial broiler chicken. J. Anim. Res., 7: 717-721.

19. Gilliland, S.E. (1989) Acidophilus milk-products a review of potential benefits to consumers. J. Dairy Sci., 72: 2483-2495.

20. Huang, Y. and Zheng, Y. (2010) The probiotic Lactobacillus acidophilus reduces cholesterol absorption through the down-regulation of Niemann-Pick C1-like 1 in Caco-2 cells. Br. J. Nutr., 103: 473-478.

21. Surono, I.S. (2003) In vitro probiotic properties of indigenous dadhi lactic bacteria. Asian Aust. J. Anim. Sci., 16: 726-731.

22. Kalavathy, R., Abdullah, N., Jalaludin, S. and Ho, Y.W. (2010) Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br. Poult. Sci., 44: 139-144.

23. Al-Saad, S., Abhob, M. and Abo Yones, A. (2014) Effect of some growth promoters on blood hematology and serum composition of broiler chickens. Int. J. Agric. Res., 9: 265-270.

24. Ashayerizadeh, O., Dastar, B., Samadi, F., Khomeiri, M., Yamchi, A. and Zerehdaran, S. (2014) Comparison between the effects of two multi-strain probiotics and antibiotics on growth performance, carcass characteristics, gastrointestinal microbial population and serum biochemical values of broiler chickens. Sci. J. Anim. Sci., 3(4): 110-119.

25. Abeer, E.S.M. and Mosaad, S.A. (2015) Effect of dietary probiotic and/or prebiotic supplementation on growth performance, carcass traits and some serum biochemical alterations in broiler chicken. J. Anim. Sci. Adv., 5(11): 1480-1492.

26. Haddadin, M.S.Y., Abdurahim, S.M., Hashlamoun, E.A.R. and Robinson, R.K. (1996) The effect of Lactobacillus acidophilus on the production and commercial composition of hen’s egg. Poult. Sci., 66: 480-486.

**********