Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2019; 7(5): 141-145

Received: 21-07-2019
Accepted: 25-08-2019

Muralidhar S Talkad
Animal Health Science, R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City PO, Hosur Road, Bengaluru, Karnataka, India

Prashanth D'souza
Animal Health Science, R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City PO, Hosur Road, Bengaluru, Karnataka, India

Saravanakumar Marimuthu
Animal Health Science, R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City PO, Hosur Road, Bengaluru, Karnataka, India

Corresponding Author:
Saravanakumar Marimuthu
Animal Health Science, R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City PO, Hosur Road, Bengaluru, Karnataka, India

Small intestinal gut-associated lymphoid tissue histomorphometry analysis in broilers supplemented with Stodi®

Muralidhar S Talkad, Prashanth D'souza and Saravanakumar Marimuthu

Abstract
The present study was conducted with objective to evaluate the effect of Stodi® on ileum and Meckel's diverticulum gut-associated lymphoid tissue (GALT) proliferation by histomorphometry analysis. 960 one-day-old broiler chicks (Ross 308) were divided into four groups: normal control group, negative control group and two Stodi® treatment groups (500 g/ton and 750 g/ton). All the groups except normal control were challenged with $MgCl_2.6H_2O$ to increase the cecal moisture content and subsequently to disturb the cecal microbiota. At day 42, samples were taken from different parts of small intestine (ileum and Meckel's diverticulum) and fixed in 10% buffered formaldehyde (pH 7.4). Sections of 4 µm thickness were made using microtome and stained by H&E. The results of the present study revealed that area of GALT (μm^2) was significantly ($p<0.05$) increased in the lamina propria of ileum and Meckel's diverticulum in Stodi® (750 g/ton) treated group as compared to negative control. The knowledge gained from this research study is that dietary supplementation of Stodi® at 750 g/ton of feed caused proliferation of morphology and associated immune structures of GALT in both ileum and Meckel's diverticulum proving Stodi® as a potential immunomodulatory candidate to enhances the performance traits in Ross 308 broiler chickens.

Keywords: GALT, small intestine, ileum, Meckel's diverticulum, histomorphometry

Introduction
In poultry "Gut health" is an extensively debated topic of research interest in the literature of veterinary sciences. The health of gastrointestinal system helps poultry in achieving optimal production performance through two main enhanced functions of gut like digestion and absorption of nutrients and immunity. Microbiota, immune system and nutrition are the three key components of gut interdependently determines the optimal working competence of gut [1, 2]. Stable equilibrium of gut microbiota especially commensal microbiota plays a pivotal role in determining the gut health like development of gut structure and morphology, inhibition of invading pathogens, and assist in enhanced digestion and optimal absorption of nutrients in the intestine [3].

In monogastric, gastrointestinal mucosa is the first line of defense mechanism to fight with pathogens. In the intestine of the monogastric animal there has been a complex system of the submucosal and mucosal lymphatic tissue called "GALT"- gut-associated lymphoid tissue. GALT plays a pivotal role in controlling the incidence of poultry enteric disorders through its immunological functions [4, 5] as it is exposed to the microflora from concomitant feed and the environment [6]. The definable structures of GALT include lymphoid aggregates located within the lamina propria, Meckel's diverticulum, peyer's patches and cecal tonsils. GALT encompasses 75% of all lymphoid cells of the entire immune system and among them roughly 80% of all immunoglobulins (Ig) and 50% of lymphocytes are produced in the intestine. Production of IgA antibodies secreted on the mucosal surface is the hallmark feature of GALT system. Their main function involves stoppage of antigens entering organism through mucosa and this will be achieved by capture of antigens by specialized antigen presenting cells (APC) which secrete appropriate cytokines, thereby determining development or mitigation of inflammation [7, 8]. Recent research has been focused more specifically on the immunological activities of GALT, in order to explore the alternative methods of achieving target weight chickens [9].

Typically, ingested feed, environment, pathogenic microorganisms and management practices adversely affects the equilibrium balance in the components of chicken gut, which leads to enteric disease and results in sub-optimal production performance of chickens [10, 11]. In addition, these disorders of gut health are one of the factors that affects the prevalence of wet litter [12] and it has been evident from the literature that incidence of foot pad dermatitis is directly proportional to wet litter moisture content [13, 14]. Marimuthu *et al.* reported that addition of Stodi® in the commercial broiler feed causes alteration of cecal microorganisms conducive for the gut health and enhanced performance characteristics [15]. Moreover, literature survey suggests that there is a need of preliminary studies on conducive effects of supplementation of phytobiotics as feed additives on histomorphometry of intestinal GALT to witness the potent immunostimulatory effects of such phytobiotics.

Numerous models are available to evaluate the gut disturbances in broiler chickens. One of the them is that the addition of minerals at high doses in the diet can lead to diarrhea as the high levels of minerals affect the osmolarity of the intestinal content and the water reabsorption. Van der Hoeven-Hangoor *et al.* reported that addition of magnesium ions to the diet of broilers increases the digesta and excreta moisture content in a linear manner and this increase was highest for magnesium chloride, followed by magnesium sulphate and magnesium oxide [16]. With this view points and with our hypothesis of phytobiotic feed additive, Stodi®, might boost the immunological functions of GALT in broiler chickens, the present study was undertaken to evaluate the effect of Stodi® on histomorphology of small intestinal GALT in Ross 308 broiler chickens fed with magnesium chloride added diet.

Material and Methods

Polyherbal formulation

Stodi® is a proprietary polyherbal formulation developed by M/s. Natural Remedies Pvt. Ltd., Bengaluru, India, containing a blend of *Punica granatum*, *Andrographis paniculata*, *Acacia nilotica* and *Terminalia bellirica* and *Holarrhena antidysenterica*.

Ethical approval

The study was conducted by authorized, qualified, and trained veterinarians, scientists, and technicians, in compliance with the guidelines of the Institutional Ethics Committee (IEC Number: AHS/PR/01/2018). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental setup

The study was conducted in poultry research station recognized by the Department of Scientific and Industrial Research, India; DSIR Reg No.: TU/IV-RD/2000/2016, located in Anniyalam, Tamil Nadu for a period of 42 days.

The chicks were housed in a semi-closed house divided into pens with floor space of 60 square feet. The approximate size of the individual pen was 6'×10'×5' (length × width × height). Each pen was equipped with a brooder, a bell drinker, a chick feeder, and/or jumbo feeder. The size and floor space of the pen were modified according to the number of chicks housed with the help of a polyvinyl chloride sheet. The chicks were provided with poultry mash feed *ad libitum* [17] manufactured by Higain Feeds and Farms India Pvt. Ltd., Bengaluru (Pre-starter feed with 3000 kcal metabolizable energy/kg and 21% crude protein from days 1 to 10, starter feed with 3125 kcal metabolizable energy/kg and 20% crude protein from days 11 to 24 and finisher feed with 3150 kcal metabolizable energy/kg and 18.50% crude protein from days 25 to 42) [15].

A total of 960 one-day-old broiler chicks (Ross 308) purchased from Kavi Protein and Feed Pvt. Ltd., Bengaluru, were allocated equally into four groups (6 replicates/group; 40 birds/replicate) consisting of a normal control, negative control, and treatment groups of Stodi® at two doses (500 g/ton and 750 g/ton). All the groups except normal control were challenged with 1.70% magnesium chloride hexahydrate ($MgCl_2.6H_2O$) to increase the cecal moisture content as magnesium chloride is known to decrease the reabsorption of water in the cecum and subsequently to disturb the cecal microbiota. All birds appeared healthy throughout the experimental period *i.e.* 42 days.

Histomorphological examination

At day 42, the eviscerated small intestines of 8 birds/treatment were collected. A piece (around 2 cm) of ileum (Segment from Meckel's diverticulum to cecal junction) and Meckel's diverticulum (residual tiny sac-marks the end of the jejunum and the start of the ileum) were excised and flushed with physiological saline. The collected segments were fixed in 10% buffered formalin (pH 7.4) and routinely processed in paraffin. Sections of 4 µm thickness were made using Microtome (Leica RM 2125, Leica Microsystems GmbH, Wetzlar, Germany) from each block and mounted on glass slides and stained with hematoxylin and eosin method and then mounted (DPX mutant, S. d. fine-chem Ltd., Bengaluru, Karnataka, India). The slides were examined and photographed under a light microscope (Olympus microscope) [18].

Results

It was found that area of GALT (μm^2) was significantly ($p<0.05$) increased in the lamina propria of ileum and Meckel's diverticulum in Stodi® (750 g/ton) treated group as compared to negative control (Table 1 and Fig 1 & 2). The results of the present study revealed that though both the doses of Stodi® (500 g/ton and 750 g/ton of feed) proliferated the histomorphological structure of small intestinal GALT in the lamina propria of ileum and Meckel's diverticulum of broiler chickens, but the profound effect was observed in highest dose of Stodi® (750 g/ton). The improvement was more effective on ileum part than the Meckel's diverticulum part.

Table 1: Analysis of GALT Area in Small Intestine

Area of GALT (μm^2)		
Group	**Ileum**	**Meckel's Diverticulum**
G1 - Normal Control	75368.86 ± 8945.25	57501.64 ± 5499.98
G2 - Negative Control	88360.78 ± 13011.44	45228.50 ± 9316.11
G3 - Stodi® (500g/ton)	107745.28 ± 12895.21	66035.13 ± 7138.70
G4 - Stodi® (750g/ton)	*173320.80 ± 29554.60	*84184.46 ± 11850.71

Values are expressed as Mean ± S.E.M, n=7-8; *$p<0.05$ as compared to Negative Control by one-way ANOVA followed by Dunnett's Multiple Comparison Test

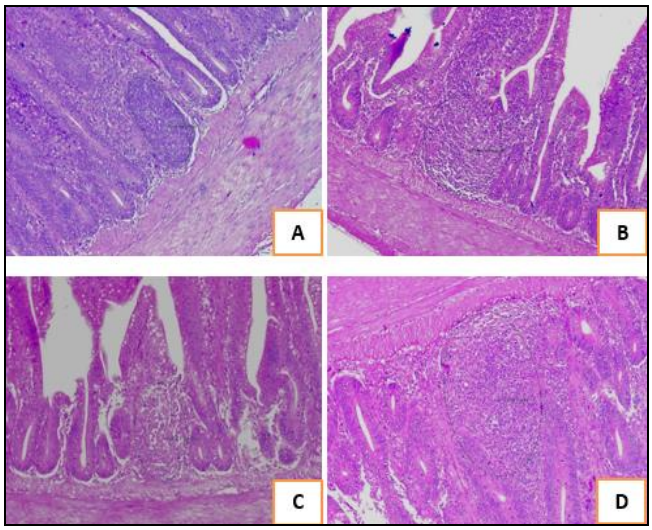

Fig 1: Photomicrographs (Optical microscopy) of haematoxylin and eosin-stained broiler ileal sections from different treatments. (A) G1-Normal control (B) G2-Negative Control (C) G3- Stodi® (500g/ton of feed); proliferation of GALT area was observed (D) G4-Stodi® (750g/ton of feed); proliferation of GALT area was observed.

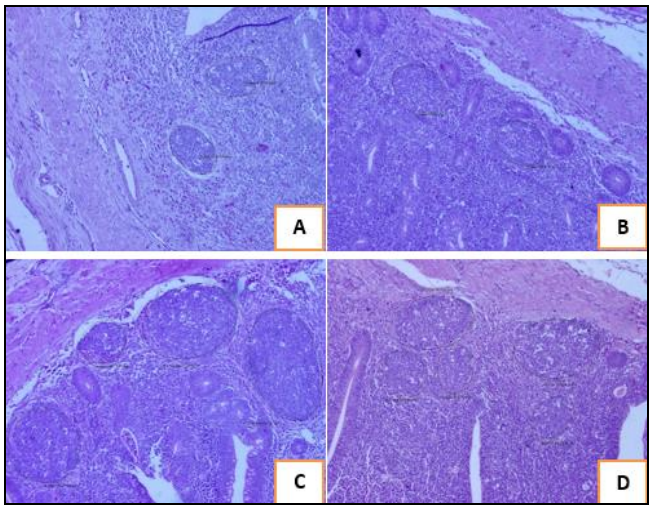

Fig 2: Photomicrographs (Optical microscopy) of haematoxylin and eosin-stained broiler Meckel's diverticulum sections from different treatments. (A) G1-Normal control (B) G2- Negative Control (C) G3-Stodi® (500g/ton); proliferation of GALT area was observed (D) G4-Stodi® (750g/ton); proliferation of GALT area was observed.

Discussion

The only alternative way to reinforce the intestinal defense mechanism is by using growth promoters like phytobiotics, probiotics and prebiotics as feed additives. Their ability to strengthen the host mucosal immunity and better resistance to invading pathogens exerts positive effect on animal health status [19]. Phytobiotics or phytogenic can be defined as plant derived products added to feed in order to improve performance through stimulation of feed intake in birds, stabilization of gastrointestinal tract microbiota and augmentation of their resistance [20-24].

In poultry, spleen-associated lymphoid tissue and GALT are the main components of lateral immune system. Payer's patches and cecal tonsils are the main GALT lymphoid aggregates in birds apart from esophageal tonsil, bursa of fabricius and localized lymphoid follicles that form near a site of infection [9]. The GALT reacts with the microflora come from the feed and the environment. Thus, there was a relationship between the intestinal microflora and the GALT [6].

In the present study, Stodi® at the dose of 750 g/ton increased the GALT surface area ($p<0.05$) in the lamina propria of ileum and Meckel's diverticulum part in chickens fed with $MgCl_2$ added diet. Sehm *et al.* in a research study on influence of polyphenol rich apple pomace or red-wine pomace diet on the gut morphology in weaning piglets reported, feeding flavonoids rich feed regimen improved the weaning piglet's health through the activation of GALT by enlargement of peyer's patches in the ileum [25]. In another study reported by Kiczorowska *et al.* supplementation of broiler diets with *Boswellia serrata* resin augmented the production performance of broiler chickens and at the same time exerted a beneficial effect on intestinal microflora and morphology [26, 27], which was further supported by other study reported by Tabatabaei [28]. Similarly, Jacobs and Pearsons reported that the supplementation of probiotics altered the cytokines secretion and lymphoid cell count in the chicken gut, this might help to enhance the immunity of chickens to *Eimeria acervulina* [29].

The literature survey disclosed that herbal constituents present in Stodi® alone or in combination are claimed to possess potent immunomodulatory effects. Nety *et al.* reported that methanolic extract of *A. paniculata* exerts potent immunomodulatory effect in broiler chickens [30]. In another study, Sunder *et al.* reported that the supplementation of extract of *A. paniculata* in broiler Japanese quail augmented the response of B cell and T cell [31]. Similarly, methanolic extract of *T. bellirica* was proven to be a potent inducement for enhanced T-lymphocyte proliferation as compared to phytohemagglutinin alone [32].

GALT fascinated the curiosity for producing defensive immunity against local and systemic pathogens [33]. In the current study, Stodi® supplementation increased the surface area of GALT in the lamina propria of ileum and more importantly GALT of Meckel's diverticulum as well. It might be an indicative of immunomodulatory effect of Stodi® on small intestinal GALT. This study provides considerable preliminary report to hint that supplementation of Stodi® as feed additive in commercial broiler feed could proliferate the histomorphology of small intestinal GALT; however detailed study needs to be conducted to better elucidate mechanism of action and ultimate impact on gut health of small intestine.

Conclusions

In conclusion, dietary inclusion of Stodi® specifically at the dose of 750 g/ton of feed during the rearing period of broilers, could improve the morphology and associated immune structures of GALT in both ileum and Meckel's diverticulum parts of small intestine in broiler chickens. This enable the organs to compete with invaded organisms helps to maintain the gut health, which in turn improves the production performance of broiler chickens. Hence, Stodi® at the dose of 750 g/ton of feed was proven as a potential immunomodulatory candidate to enhances the performance traits in Ross 308 broiler chickens.

Acknowledgements

The authors are thankful to Vittarthaa life sciences, Bengaluru, Karnataka for their lab support.

References

1. Cummings JH, Antoine JM, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC *et al.* PASSCLAIM 1—gut health and immunity. European Journal of Nutrition. 2004; 43(2):ii118-73.

2. Laudadio V, Dambrosio A, Normanno G, Khan RU, Naz S, Rowghani E *et al.* Effect of reducing dietary protein level on performance responses and some microbiological aspects of broiler chickens under summer environmental conditions. Avian Biology Research. 2012; 5(2):88-92.
3. Kogut MH, Yin X, Yuan J, Broom LJ. Gut health in poultry. CAB Reviews. 2017; 12(031):1-7.
4. Reid G, Friendship R. Alternatives to antibiotic use: probiotics for the gut. Animal Biotechnology. 2002; 13(1):97-112.
5. Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN *et al.* Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Animal Health Research Reviews. 2008; 9(2):217-225.
6. Bar-Shira E, Sklan D, Friedman A. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Developmental & Comparative Immunology. 2003; 27(2):147-157.
7. Butler JE, Sinkora M. The enigma of the lower gut-associated lymphoid tissue (GALT). Journal of leukocyte biology. 2013; 94(2):259-270.
8. Uddin MJ, Kaewmala K, Tesfaye D, Tholen E, Looft C, Hoelker M *et al.* Expression patterns of porcine Toll-like receptors family set of genes (TLR1-10) in gut-associated lymphoid tissues alter with age. Research in veterinary science. 2013; 95(1):92-102.
9. Casteleyn C, Doom M, Lambrechts E, Van Den Broeck W, Simoens P, Cornillie P. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. Avian Pathology. 2010; 39(3):143-150.
10. Hughes RJ. An integrated approach to understanding gut function and gut health of chickens. Asia Pacific Journal of Clinical Nutrition. 2005; 14:S27.
11. Dekich MA. Broiler industry strategies for control of respiratory and enteric diseases. Poultry science. 1998; 77(8):1176-1180.
12. Montagne L, Pluske JR, Hampson DJ. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal feed science and technology. 2003; 108(1-4):95-117.
13. Youssef IM, Beineke A, Rohn K, Kamphues J. Effects of litter quality (Moisture, ammonia, uric acid) on development and severity of foot pad dermatitis in growing turkeys. Avian diseases. 2011; 55(1):51-58.
14. Wu K, Hocking PM. Turkeys are equally susceptible to foot pad dermatitis from 1 to 10 weeks of age and foot pad scores were minimized when litter moisture was less than 30%. Poultry science. 2011; 90(6):1170-1178.
15. Marimuthu SK, Balasubramanian B, Selvam R, D'Souza P. Modulation of chicken cecal microbiota by a phytogenic feed additive, Stodi®: A metagenomic analysis. Pharmacognosy Research. 2019; 11(3):201-209.
16. Van der Hoeven-Hangoor E, Van de Linde IB, Paton ND, Verstegen MW, Hendriks WH. Effect of different magnesium sources on digesta and excreta moisture content and production performance in broiler chickens. Poultry science. 2013; 92(2):382-391.
17. NRC. Nutrient Requirement of Poultry. 9th revised ed. Washington, DC, USA: National Academy Press, 1994.
18. Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M, Hernandez-Santana A. Effect of dietary inclusion of a plant extract blend on broiler growth performance, nutrient digestibility, caecal microflora and intestinal histomorphology. International Journal of Poultry Science. 2017; 16(9):344-353.
19. Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L *et al.* Antibiotic alternatives: the substitution of antibiotics in animal husbandry?. Frontiers in microbiology. 2014; 5(217):1-15.
20. Frankic T, Voljg M, Salobir J, Rezar V. Use of herbs and spices and their extracts in animal nutrition. Acta Agric Slov. 2009; 94(2):95-102.
21. Yang Y, Iji PA, Choct M. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World's Poultry Science Journal. 2009; 65(1):97-114.
22. Huyghebaert G, Ducatelle R, Van Immerseel F. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal. 2011; 187(2):182-188.
23. Iranparast F, Parsaei S, Houshmand M, Naghiha A. The effect of oral consumption of guggul (*Commiphora mukul*) resin on performance and humoral immunity response of broilers. Int. J. Adv. Biol. Biom. Res. 2014; 2:802-810.
24. Kumar M, Kumar V, Roy D, Kushwaha R, Vaiswani S. Application of herbal feed additives in animal nutrition-a review. Int J Livest Res. 2014; 4(9):1-8.
25. Sehm J, Lindermayer H, Dummer C, Treutter D, Pfaffl MW. The influence of polyphenol rich apple pomace or red-wine pomace diet on the gut morphology in weaning piglets. Journal of animal physiology and animal nutrition. 2007; 91(7-8):289-296.
26. Kiczorowska B, Samolińska W, Al-Yasiry AR, Kowalczyk-Pecka D. Effect of *Boswellia serrata* dietary supplementation on growth performance, gastrointestinal microflora, and morphology of broilers. Annals of animal science. 2016a; 16(3):835-849.
27. Kiczorowska B, Al-Yasiry AR, Samolińska W, Marek A, Pyzik E. The effect of dietary supplementation of the broiler chicken diet with *Boswellia serrata* resin on growth performance, digestibility, and gastrointestinal characteristics, morphology, and microbiota. Livestock Science. 2016b; 191:117-124.
28. Tabatabaei SN. Effect of olibanum (*Boswellia thurifera*) as a feed additive on performance, some blood biochemical and intestinal morphology in broiler chicks. Research Opinions in Animal and Veterinary Sciences. 2016; 6(4):130-134.
29. Jacobs CM, Parsons CM. The effect of Grobiotic-P combined with yeast cell wall and gluconic acid on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. Poultry science. 2009; 88(11):2360-2367.
30. Nety S, Koley KM, Chourasia D, Sharma K, Bhandeker SK. Study of Phytochemical and Immunomodulatory Activity of Methanolic Extract of *Andrographis paniculata* in Broiler Birds. Journal of Animal Research. 2018; 8(1):27-31.
31. Sunder J, Sujatha T, Rajas A, Kundu A. Immunomodulatory effect of *Morinda citrifolia* and *Andrographis paniculata* on expression of toll-like receptors in Nicobari fowl. Indian J. Anim. SCI. 2016; 86:1006-1008.
32. Hu ZQ, Toda M, Okubo S, Hara Y, Shimamura T. Mitogenic activity of (−) epigallocatechin gallate on B-cells and investigation of its structure-function

relationship. International journal of immunopharmacology. 1992; 14(8):1399-1407.
33. Revolledo L, Ferreira AJ, Mead GC. Prospects in Salmonella control: competitive exclusion, probiotics, and enhancement of avian intestinal immunity. Journal of Applied Poultry Research. 2006; 15(2):341-351.
34. Ibrahim F Rehan, Hesham H Mohammed, Sohaila Gehad Fahmy, Asmaa Elnagar, Mohammed Youssef, Obeid Shanab. Influence of photoperiod and circulating-IgY on some behavioural patterns of chicks during the first week of life. Int J Vet Sci Anim Husbandry 2019;4(2):18-25.