Duodenal Histomorphological Changes in Broilers Administered poly d, l-lactic-coglycolic acid (PLGA) Nanoparticles Encapsulated with Peptide

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ABSTRACT

This research was carried out to evaluate the effect of poly d, l-lactic-coglycolic acid(PLGA) nanoparticles loaded with peptide (as vaccine) on histomorphological in duodenum of broiler chick. A total of ninety eight, one day old ,unsexed broiler(Rose) chicks were divided randomly into seven groups(2 replicate in each group) as follows:G1-control,G2 - chicks received traditional vaccine of infectious bursal disease(Volvac[@] IBD MLV) ,G3 – Chicks Received PLGA nanoparticles only, G4,G5,G6 and G7 – chicks were received prepared vaccine at 160, 80, 40,and 20 µg of peptide loaded PLGA respectively. At the end of the experiment histopathological examination of duodenum section and histomorphological changes were examined. The histopathological examination of duodenal sections shows an elongation and infiltration of lamina propria (LP) with increase villi height and crypt depth in groups that received PLGA alone and those received peptide loaded PLGA. At the same time these groups reveal an increase in mucosal thickness and in length and width of villi. The histomorphological examination in this study show thicker mucosal layer with deeper crypt in duodenum of broilers that subjected to PLGA administration alone or to different concentration of peptide loaded PLGA compared to G1 and G2. These findings are accompanied by a high density of goblet cells and lower villus hight/crypt depth (V/CD) ratio. All groups show mononuclear cells (MNCs) infiltration in submucosa ofduodenum. In-conclusion, the administration of PLGA nanoparticles is strongly linked to the improvement of the physiological and immunological features of the birds.

Keywords: PLGA nanoparticls, Broilers, Duodenum, Histopathological changes

Introduction

The field of polymer nanoparticle (PNP) is rapidly growing and playing a necessary role in a varied field of area extending from medicine to biotechnology, conducting material to sensors, electronics and so forth (1,2**)**. Polymers are the most common materials for erecting nanoparticle-based drug carriers. In 1979 and for the first time the polymer polyalkylcyano acrylate nanoparticle was studied as absorption of anticancer drugs (3). Biodegradable nanoparticles (NPs) (diameter: 10 to 1000 nm) are acquisition increased attention because of their ability to avail as a viable carrier for delivery of drugs and vaccine, which serving as excellent carriers by promoting solubility, increasing clearance half-life and targeting drug to specific sites in the body and have been proved to enhance the oral bioavailability of oral inactive antibiotics (4).

A different type of synthetic polymers are used in preparing nanoparticles, such as poly (d,l-lactide-co-glycolide) (PLG) (5), poly(d, l-lactic-coglycolic acid)(PLGA)(6), poly(ethylene glycol) (PEG), poly (g-glutamic acid) (g-PGA) (7),and polystyrene (8). PLG and PLGA nanoparticles are the most widely investigated because of their biocompatibility and

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Department of Agricultural Research, Ministry of Science and Technology, Iraq. Received: 15 Jun 2019, Accepted: 5 December 2019, Published: 28 Jun 2020. This article is an open access article under the terms and conditions of the Creative Commons Attribution License (CC BY 04).
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DOI: https://doi.org/10.30539/ijvm.v44i1.945

biodegradability (9). These nanoparticles entrap antigen for delivery to specific cells or maintain antigen release because of their slow degradation rate (10).

Poly (lactide-co-glycolide) (PLGA) is asynthetic copolymer of lactic acid (a-hydroxypropanoic acid) and glycolic acid (hydroxyl acetic acid) and one of the best regularly degradable polymers. PLGA has generated great interest because of its astonishingbiodegradability, biocompatibility, and mechanical strength (11).Thus,this experiment was designed to evaluate the effect of PLGA as adjuvant on histopathological and histomorphological changes of duodenum.

Materials and Methods

Preparation of PLGA: PLGA nanoparticles encapsulating peptide were prepared by solvent evaporation method (12, 13), with some modification, which can be summarized: 200 mg of PLGA dissolved in 2 ml of DMSO and left at room temperature overnight, PVA prepared by dissolving 2gr in 100 ml of diH2O. The PLGA solution added drop wise to PVA and left overnight on stirrer then the produced solution was centrifuged and wash 3X and the precipitate was collected and lyophilized for 72hr.

In this study, 98 one day old, broiler chick (Ross-308) obtained from a commercial hatchery, Suhool Al-Khairat, Babil province were used, randomly divided equally into 7 groups (14 chick) fed on an ordinary diet, starter from day 1 to 20 (22% crude protein, 2926 K Cal/Kg) and finisher from day 21 to 42 (19% crude protein, 3109 K cal/Kg) *ad libitum*. On day 19 the broiler chick administered orally with distilled water (G1), Traditional vaccine (G2), PLGA NPs (G3), PLGA+160µg peptide(G4), PLGA+80µg peptide(G5), PLGA+40µg peptide (G6) and PLGA+20µg peptide(G7).

At the end of study 2 broiler from each replicate were sacrificed and duodenum collected and after hematoxylin/eosin staining of series section of different parts of the intestinal wall, as described by(14), the mucosal thickness, villus hight, crypt depth and villus hight/crypt depth ratio were measured at low power (10 X) using a Winjoe ocular micrometer.Also, goblet cells were identified as acidic mucin secreting cells and were assessed by staining with periodic acid Schiff (PAS), according to (15). Determination of the number of goblet cells limited by cells with PAS positive stained along the villi by the light microscope, as cell/100 enterocyte. Goblet cells were measured in twenty fields for each intestinal sample/animal. Only the goblet cells found on the edge of the villus were counted in the density determination.

Statistical Analysis

Statistical analysis was performed using SAS (Statistical Analysis System version 9.1. One-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among means. ($P \leq 0.05$) was considered statistically significant (16).

Results and Discussion

The results of our experiment in regard to histomorphological parameters (mucosa thickness, villus hight, crypt depth, and villus/crypt ratio) of the duodenum piece in response to ordinary vaccine and PLGA nanoparticles and PLGA loaded with different concentration of peptide (antigenic region) are illustrated in Figure (1, 2, 3,and 4).

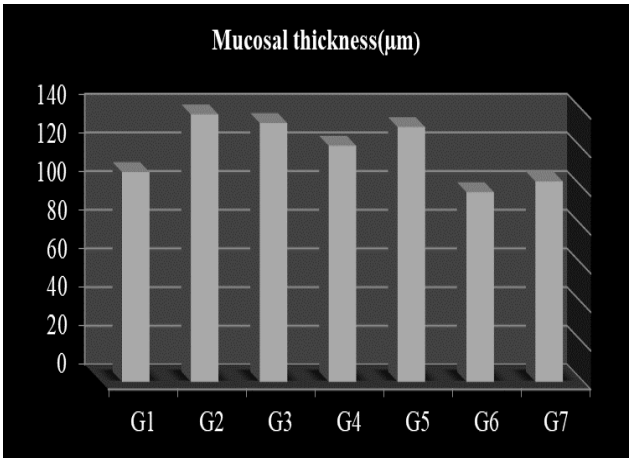


Figure 1. Effect of PLGANpsand PLGA loaded with different concentration of peptideon mucosal thickness (µm). G1=received diH2O, G2= ordinary vaccine, G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide,G7=PLGA+20 µg peptide

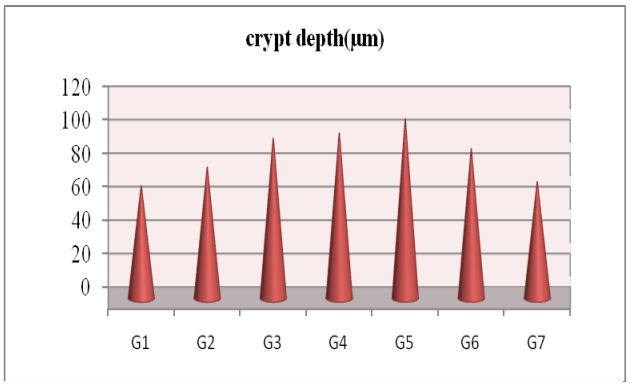


Figure 2. Effect of PLGANpsand PLGA loaded with different concentration of peptideon villus hight(µm). G1=received diH2O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide, G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide

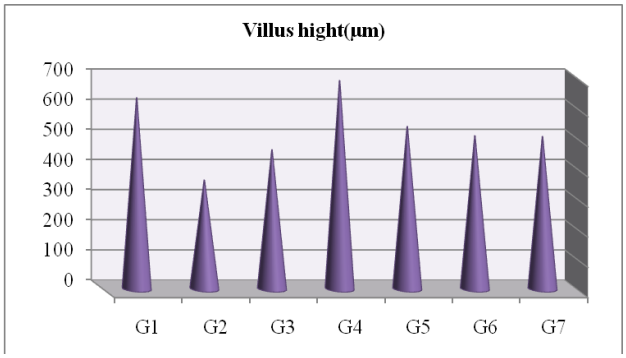


Figure 3. Effect of PLGA Npsand PLGA loaded with different concentration of peptide on crypt depth(µm). G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle, G4= PLGA+160 µg peptide,G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide

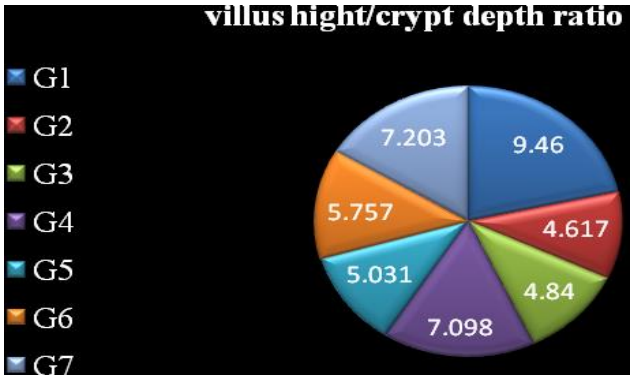


Figure 4. Effect of PLGA Npsand PLGA loaded with different concentration of peptide on villus hiegh/ crypt depth ratio. G1=received diH2O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide.

cular,small intestine mucosa plays an important role in absorption. Villus length, crypt depth and villi/crypt ratios are important indicators of intestinal morphology, which play critical roles in nutrient absorption (17). The small intestine is the place for digestion and absorption of nutrients, and the intestinal mucosa plays an important role in these processes (18). In the present study, oral administration of NPs +160 µg of peptide led to increase villus length, which would be useful for absorbing of intestinal nutrients.

The mucosal surfaces of small intestine are lined by epithelial cells, which are derived from self-renewing stem cells that reside at the crypt base (19). In our present study, oral administration of NPs+80µg of peptide increased crypt depth in duodenum, which suggests the ability of intestinal stem cells for self-regeneration and proliferation might have been enhanced (20). The ratio of villus height to crypt depth is a useful standard for estimating the digestive capability in the small intestine (21). Increased villus height and villus surface area are associated with greater absorption of available nutrients (22). In fact, long villi arecorrelated with better gut health(23). In present study, the possible explanation for higher villus height may be due to higher bioavailability of nanoparticles, so maintaining epithelial barrier integrity and function (20), reducing the turnover rate of cells in the villiand resulting in higher villus height.

Our obtained results agreed with finding of Xu*et al.* (18) who used polymeric NPs in weanling piglets. Similar results were also reported by another investigator (24). Also, supplementation of non-organic NPs (nano-ZnOs) significantly increased villus length, crypt depth, villus width, and villus surface area ($P \leq 0.05$), while had no effects on villi/crypt ratio ($P \geq 0.05$) (19, 25).

From Histopathologic examination of duodenum section of broilers G1(diH2O), with H &E stain, shows normal architecture (mucosal thickness, villus height, crypt depth) (figure,5A) with mild infiltration of mononuclear cells (MNCs) in submucosa. The duodenum section of G2(traditional vaccine) (Figure,5B) shows enlarged and thickness of mucosa with infiltrations of MNCs and

increased size of mucus glands. On the other hand, G3(NPs) section (Figure 5C) showed elongation of villi with increase mucosal thickness and focal aggregation of lymphocytes in submucosa. G4 (NP + 160 µg peptide) duodenum section in (Figure5D) show significant ($p \leq 0.05$) increase villi height and crypt depth with infiltration of MNCs in submucosa and hyperplasia of goblet cells (GCs). In G5 (NP + 80 µg peptide) Figures(5E) show significant($p \leq 0.05$) increase in mucosal thickness with increase in crypt depth with infiltration of MNCs in submucosa and hyperplasia of GCs. G6(NP + 40 µg peptide) section (Figure 5F) show long and wide villi with infiltration of LP with MNCs and mild increase in GCs. Finally G (NP + 20 µg peptide) increase in GCs (Figure 5G).

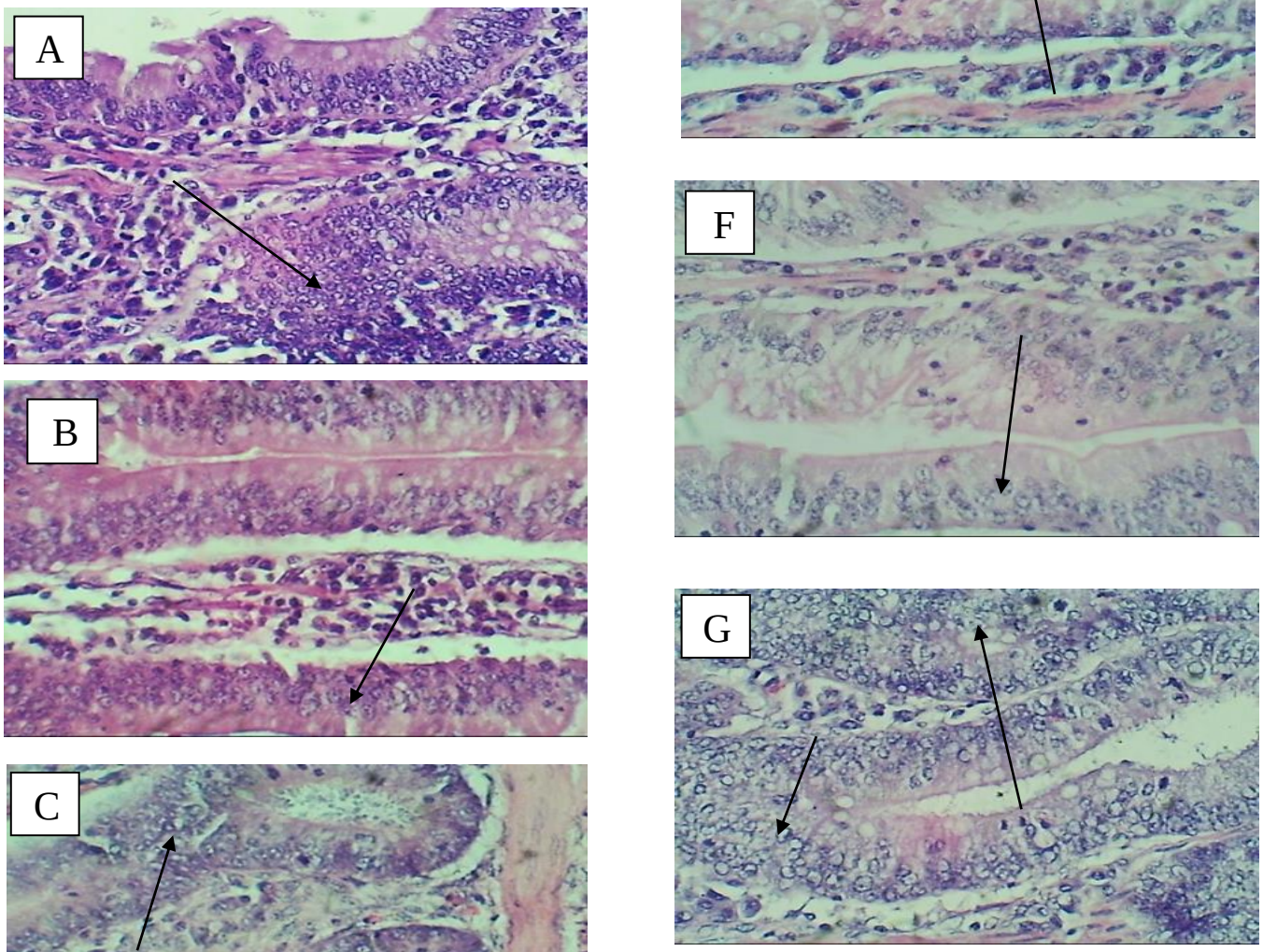


Figure 5. Light microscopic image of duodenum cross section histopathological and histomorphological changes in A(G1),B(G2),C(G3),D(G4),E(G5),F(G6), and G(7) the arrow indicates MNCs infiltration

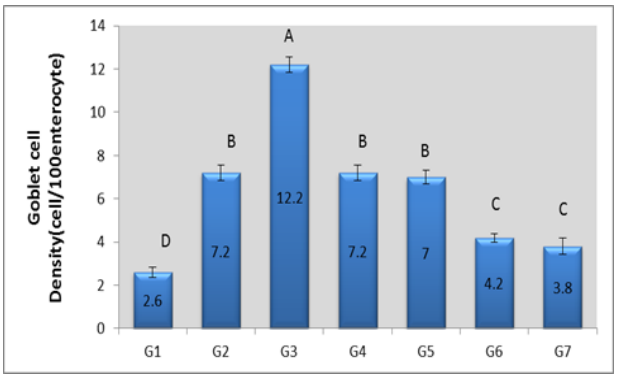
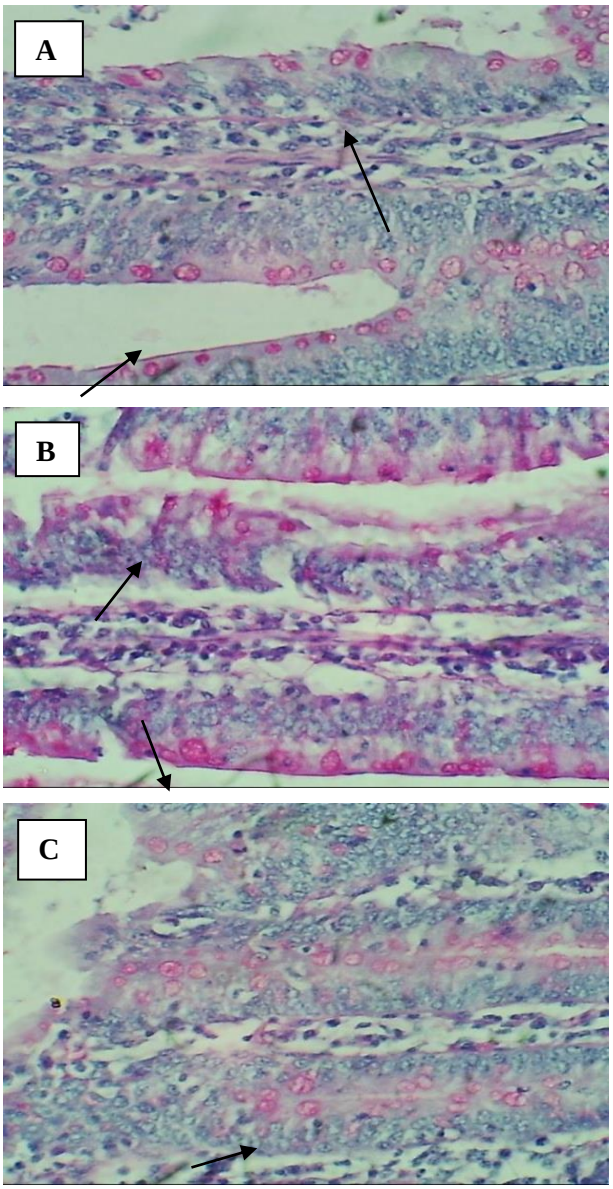


Figure 6. Effect Of PLGA Nps And PLGA Loaded with Different Concentration Of Peptide On goblet cell density (%).G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle, G4= PLGA+160 µg peptide, G5=PLGA+80µg peptide, G6=PLGA+40µg, G7=PLGA+20µg peptide peptide



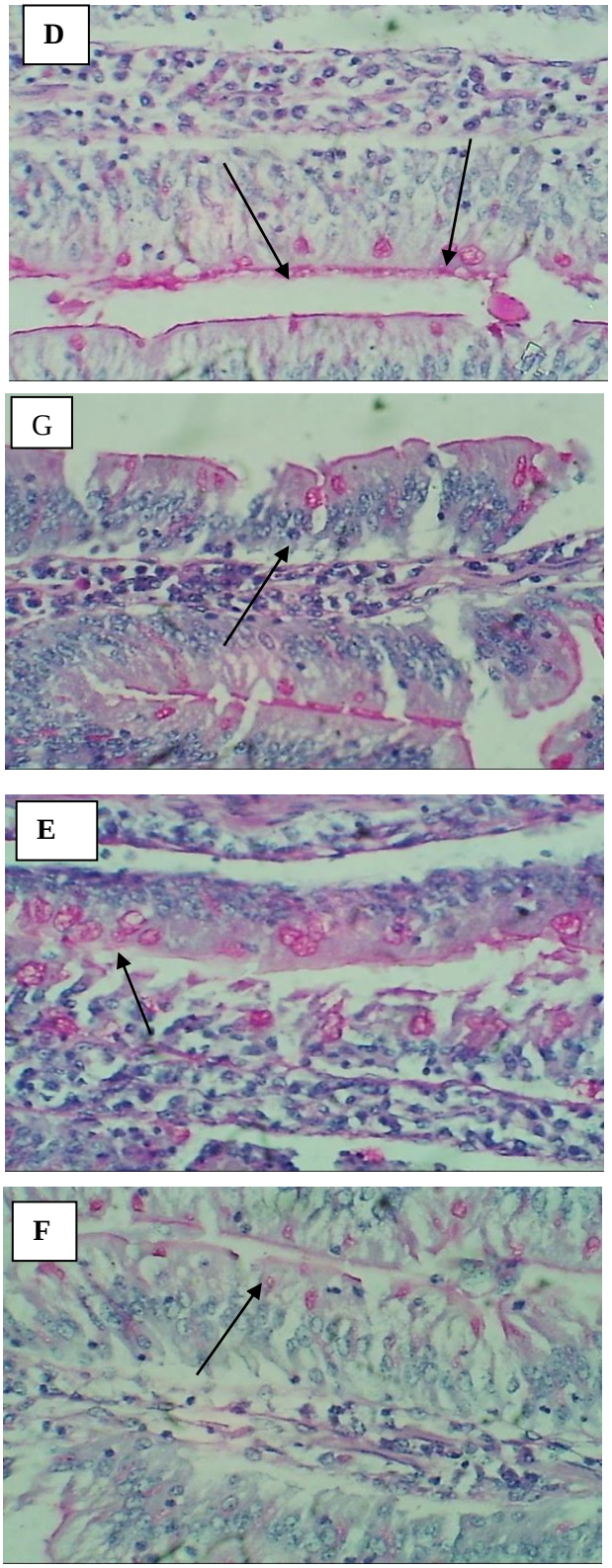


Figure 7. Light microscopic image of duodenum cross section, positive reaction color of goblet cells appeared red color with PAS in A(G1), B(G2), C(G3),D(G4), E(G5),F(G6) and G(G7).The arrow indicates to goblet cells

From the results of the histological examination of the duodenum sections, it is noticed that the tissue of all the groups were normal and have no problems. This may be since PLGA has no toxic effects on the tissues of the body (26). Also, polymeric NPs provided a beneficial environment for the proliferation of enterocytes, preventing intestinal atrophy (24). The infiltration of MNCs may be return to the role of PLGA and peptide (antigenic region) in stimulating local and systemic immune response and that agreement with Shakweh *et al.* (27).

The data obtained in our experiment demonstrated in Figure (6) pointed that the higher density of goblet cells was in G3 (PLGA NPs) as a percentage ratio to enterocyte (12.2%) followed by G2 and G4 which recored (7.2%), G5 (7%), G6(4.2%), G7(3.8%) and the lowest value recorded by G1(2.6%) while Figure (7) Light microscopic image of duodenum cross section , positive reaction color of goblet cells appeared red color with PAS.

The small intestinal epithelia present a large absorptive surface that mainly consists of absorptive enterocytes and, to a lesser extent, of mucus secreting goblet cells. The fraction abundance of goblet cells has been estimated at 10–20%, increasing towards the colon (28). Goblet cells are produced in the crypts of intestinal tract and over a period of approximately three days then these goblet cells migrate up along the sides of the villi, towards the villi tip where they will eventually be sloughed and released into intestinal lumen, these goblet cells are replaced in continuous manner (29). Goblet cells establish throughout the gastrointestinal (GI) tract and are responsible for the production and preservation of a protective mucus blanket (30). Also, intestinal goblet cells participate in maternal antibody protection as they provide a protective dam for maternal IgA antibodies prior to hatch, and in adult chickens they store endogenously derived IgA (31).

The higher acidic goblet cells could be another reason for higher villus height in the aforesaid intestinal segment, as acidic machine is resistant to bacterial degradation resulting in less cellular damage (25).

Acidic making plays a protective role against pathogen while the mixed neutral machine facilitates movement of feed due to less viscosity (32). The higher number of goblet cells due to PLGA NPs supplementation in the duodenum, may be indicate protection against pathogen, but at the same time facilitation for feed transit towards site of absorption. The increase in GC count may be indicating that PLGA NPs accelerated maturation cycle of GC. These observed effects could be attributed to higher capabilities of immuno-stimulation of PLGA NPs due to their small size and up regulation of mucin gene (25).

From the available results and the fact that the role of goblet cells in the gastrointestinal tract immunity of poultry, it is denoted that the increase in the number of goblet cells in the intestine of birds that administered nanoparticles is strongly linked to the improvement of the physiological and immunological features of the birds of the current study.

Acknowledgments

The authors would like to appreciate the college of Veterinary medicine, University of Baghdad for providing all facilities of completion this study.

Conflict of Interest

The authors declare there is no conflict of interest.

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التغييرات النسيجية للاثني عشري في افراخ فروج اللحمالمجرعة بمادة PLGA النانوية والمحمل عليها ببتيد

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الخلاصة

أجري هذا البحث لتقييم تأثير الجسيمات النانوية PLGA المحملة بالببتيد (كلقاح) على التغيرات النسيجية في الاثني عشر في افراخ دجاج اللحم. تم تقسيم 98 فرخ لحم بعمر يوم واحد وغير مجنسة بشكل عشوائي إلى سبع مجموعات (مكرران في كل مجموعة) على النحو التاليG1-control :؛ - G2 جرعتالافراخ لقاحًا تقليديًا(Volvac @ IBD MLV) ضد مرض التهاب جراب فابريشيا المعدي، - G3الافراخجرعتبجزيئات PLGAالنانوية فقط ، G4 ، G5 ، G6وG7 جرعت الافراخ PLGA المحمّل بالببتيد160 و 80 و 40 و 20 ميكروغرام على التوالي. في نهاية التجربة تم اجراء الفحص النسيجي للاثني عشر والتغيرات النسيجية. يُظهر الفحص الهستوبوبولوجيلمقاطع الاثني عشرية تثخن في الطبقة المخاطية (LP) مع زيادة في ارتفاع الزغابة وعمق السرداب في المجموعات التي جرعت PLGA وحدها و تلك التي جرعت بPLGA المحملة بالببتيد. وفي نفس الوقت اضهرت هذه المجموعات زيادة في سمك الغشاء المخاطي وطول وعرض الزغابات. يظهر الفحص النسيجي في هذه الدراسة سماكة الغشاء المخاطي أعلى مع سرداب أعمق في الاثني عشر في الافراخ التي جرعتبمادة PLGA وحدها أو لتركيز مختلف منالببتيد المحمل على PLGA مقارنة G1 و G2. ويصاحب هذه النتائج كثافة عالية من خلايا الكأس(goblet cell) وانخفاض نسبة ارتفاع الزغابة / عمق السرداب(VH / CD) . جميع المجموعات تظهر ارتشاح للخلايا وحيدة النواة في الطبقة تحت المخاطية للاثني عشر.

الكلمات المفتاحية : PLGAالنانوي, افراخ اللحم, الاثني عشر, التغيرات النسيجية .