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Effects of *Lactobacillus rhamnosus* probiotic supplementation on gut health in rabbits

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ABSTRACT

Aim: The research examined effects of prolonged oral *Lactobacillus rhamnosus* intake on rabbit intestinal tissue morphology.
Methods: The study included twelve male rabbits aged 10–12 months and weighing 1.5–2.0 kg who were randomly assigned to two groups of six animals each. The control group received distilled water but the experimental group received *L. rhamnosus* orally at 0.25 mL/kg/day for 45 days. The research team performed histological and morphometric analysis on duodenal samples to measure villus height and width and crypt depth and villus area and VH/CD ratio and muscularis thickness through ImageJ software.
Results: The extended probiotic treatment caused villus disorganization and epithelial necrosis and villus fusion and inflammatory infiltration and submucosal oedema. The quantitative analysis showed that all measured parameters including villus height and VH/CD ratio and villus area and muscularis thickness demonstrated significant decreases ($p<0.05$) when compared to the control group. The results show that absorptive capacity was impaired.
Conclusion: The duodenal mucosa developed structural and functional changes because of extended exposure to high *L. rhamnosus* concentrations, which indicates that long-term probiotic use could be harmful. Research needs to determine the correct dosage amounts and duration of treatment for non-rodent species.
Keywords: duodenum, histopathology, *Lactobacillus rhamnosus*, morphometry, probiotics, rabbits.

INTRODUCTION

The definition of probiotics includes live microorganisms which provide health advantages to hosts when taken in sufficient amounts to support intestinal microbial equilibrium and strengthen mucosal defence systems (1,2). The therapeutic potential of Lactobacillus Bifidobacterium and Saccharomyces genera bacteria has become increasingly important for treating digestive and metabolic and immune system disorders (3,4). Research studies show that probiotics enhance epithelial barrier strength and create immune tolerance through their production of short-chain fatty acids and bacteriocins which fight pathogenic bacteria (5–7). Research has focused on Lactobacillus rhamnosus because it shows excellent resistance to stomach acid and bile and strong ability to attach to intestinal cells and control inflammatory cytokines which help maintain both gut and body equilibrium (8,9). Research shows probiotics provide immediate health advantages yet studies show that taking high doses of probiotics for extended periods can harm gut bacteria and intestinal walls, which results in inflammation and reduced nutrient absorption (10–12). Most of the current literature is limited to rodent models (7), with scarce information on non-rodent species such as rabbits, which possess unique gastrointestinal physiology characterized by high microbial fermentation and susceptibility to dysbiosis (11). This gap limits the extrapolation of existing probiotic safety data to broader mammalian systems.

The aim of this study was to evaluate the histopathological and morphometric alterations in the duodenal mucosa of rabbits following 45-day oral supplementation with *Lactobacillus rhamnosus*, in order to assess potential risks associated with long-term probiotic use in non-rodent models.

MATERIALS AND METHODS

Materials and study design

The experiment was conducted at the Department of Basic Dental Sciences, University of Mosul, Iraq, from January to March 2025.

Twelve male rabbits that are healthy and domestic (*Oryctolagus cuniculus*) between 10 and 12 months old and weighing 1.5 to 2.0 kg each were kept in separate housing units with

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controlled environmental settings (temperature maintained at 22 - 25 °C and relative humidity at 50 to 60%).

Animals were provided standard rabbit chow and water ad libitum. Following acclimatization, rabbits were randomly assigned to two groups (N=6 per group): Group I (Control) obtained distilled water orally (vehicle) daily for 45 days, and Group II (Probiotic-treated) received oral drops of *Lactobacillus rhamnosus* (Vitron Farma, Turkey) at a dose of 0.25 mL/kg/day (equivalent to approximately 5 drops) for 45 consecutive days (10).

The experimental protocol was reviewed and approved by the Scientific and Ethical Committee of the Basic Dental Sciences Department at the College of Dentistry, in Mosul University, which granted approval under the reference number UOM. Dent25\/1076.

Methods

At the end of the experimental period, rabbits were euthanized humanely under appropriate anaesthesia. Duodenal segments (~0.5 cm^3) were excised, rinsed in phosphate-buffered saline, and fixed in 10% neutral buffered formalin for 24 hours. Standard histological procedures, dehydration in graded alcohols were followed: the tissue samples were prepared by clearing them with xylene and then embedding into paraffin wax before being sliced into 5 µg sections for staining with haematoxylin and eosin (H&E) (9).

Histological examination was carried out using a microscope (Olympus CX43) which had a digital camera for capturing images of the samples from the duodenal tissue of each rabbit animal under study. Five distinctive sections were prepared and stained with H&E. A total of three high power fields (HPFs) from each stained slide per rabbit were selected for detailed morphometric analysis; this resulted in 15 measurements being taken for each individual animal to provide a comprehensive overview of the mucosal architecture. The selection of these fields was made to ensure a representation of the mucosal structure, within the samples studied. Various measurements, such as the height of the villi and crypts well, as the ratio of villus height to crypt depth were calculated by utilizing Image J software (10) from the National Institutes of Health (NIH) in the United States.

Prepared slides were examined under a light microscope by a qualified histopathologist blinded to the group allocation. The histopathological‒examination revealed multiple important findings, which included villus structure assessment and evaluation of epithelial health and presence of inflammation and submucosal swelling and gland tissue damage.

Morphometric measurements were conducted using Image-Pro Plus software (version 6.0, Media Cybernetics, Rockville, MD, USA). For each animal, ten well-oriented villi and ten crypts were analysed. The assessment included measurements of villus height (VH) and width (VW), crypt depth (CD), VH/CD ratio, villus area, and muscular thickness.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical differences between the control and probiotic-treated groups were evaluated using an independent samples t-test. The research established $p \leq 0.05$ as its statistical significance threshold.

RESULTS

The duodenal sections from both experimental groups showed different structural patterns after 45 days of treatment through histological examination. The duodenal tissue in the control group maintained its typical structure because it showed well-organized villi which contained columnar epithelial cells and visible goblet cells. The submucosa and muscularis layers showed no signs of inflammation or disruption while maintaining their compact structure. The serosa displayed its typical four-layered pattern which makes up the intestinal wall structure.

The probiotic-treated group showed distinct pathological changes. The microscopic examination showed that villus structure became disorganized while their tips became flattened and adjacent villi merged together. The tissue showed two main patterns of damage which included dead epithelial cells and inflammatory cells that entered the lamina propria and submucosa. The tissue showed two main findings that included pronounced submucosal oedema and focal necrosis of enteric glands suggesting mucosal damage from extended contact with *Lactobacillus rhamnosus* (Figure 1 A–G).

The quantitative morphometric analysis verified all findings which were observed through histological examination. The probiotic-treated rabbits showed a substantial decrease ($p<0.05$) in villus height and area and VH/CD ratio and muscularis layer thickness but villus width and crypt depth remained unchanged ($p>0.05$). The research shows that probiotic treatment over time results in smaller absorptive areas and damaged mucosal tissue (Figure 2).

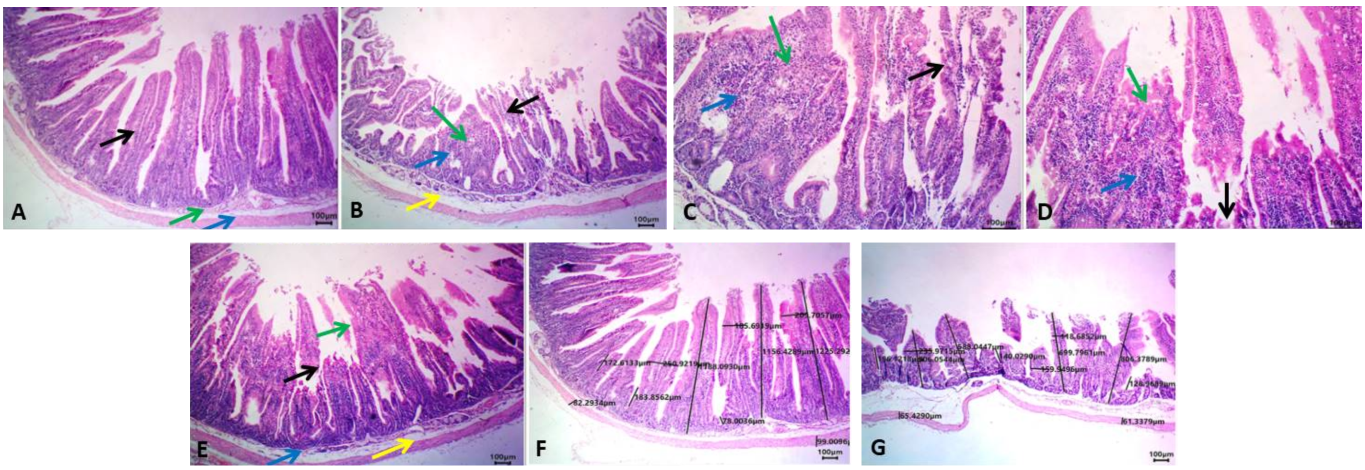

Figure 1. Photomicrographs of rabbit duodenal mucosa showing histological differences between control and probiotic-treated groups. A-B) Control group demonstrating intact villi and organized mucosa; C-G) Probiotic groups (T1–T2) showing villus disorganization, epithelial necrosis (black arrows), villus fusion (green arrows), inflammatory infiltration (blue arrows), and submucosal oedema (yellow arrows) (H&E stain; 40×–400×; scale bar = 100 µm)

Table 1. Histomorphometric parameters of the duodenum in control and probiotic-treated rabbits

Parameter	Mean±SD		p
	Control Group (N=6)	Probiotic Group (N=6)	
Villus height (VH) (µm)	1191.2±53.2	741.2±109.9	< 0.001
Villus width (VW) (µm)	197.8±31.8	163.4±55.1	0.262
Villus area ($µm^2$)	209411±27323.9	131312.6±11974.6	< 0.001
Crypt depth (CD) (µm)	129±46.6	157.2±39.6	0.333
VH/CD ratio	8.1±1.2	5.2±1.1	0.007
Muscularis thickness (µm)	113.2±22.3	61±4.3	< 0.001

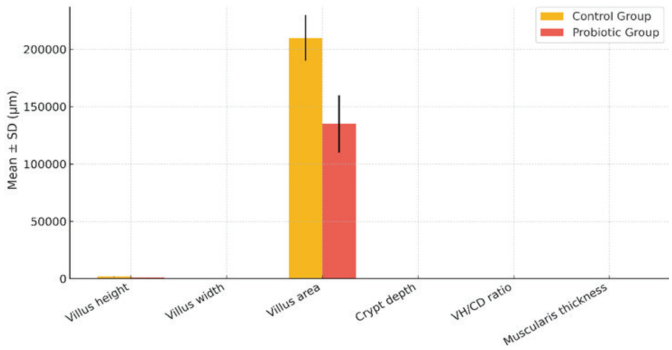

Figure 2. Quantitative comparison of duodenal histomorphometry parameters in control and probiotic-treated rabbit groups

DISCUSSION

The research demonstrates through experimental methods that rabbits who take *Lactobacillus rhamnosus* orally for long periods develop particular changes in their duodenal tissue structure and tissue appearance. The research shows that probiotics create beneficial effects on intestinal homeostasis but scientists have discovered new potential negative effects of extended and unsupervised probiotic intake. The probiotic-treated rabbits exhibited villus disorganization, epithelial necrosis, fusion of adjacent villi, inflammatory cell infiltration, and submucosal oedema—features indicating compromised mucosal integrity and the initiation of localized inflammation.

The structural changes at the mechanistic level result from disrupted tight-junction protein expression of occludin and claudins which control intestinal permeability. The disruption causes paracellular leakage and bacterial translocation resulting in immune activation through the process known as "leaky gut" (13). The tissue damage becomes more severe because inflammatory cells move into the area while TNF-α and IL-6 cytokines production increases (5,14). The study demonstrated that high probiotic doses, together with microbial imbalances, result in increased ROS production thus leading to oxidative stress and cell death of epithelial cells that prevents mucosal tissue repair (15).

The three mechanisms which disrupt tight-junction proteins occludin and claudin and cause oxidative stress and cytokine-driven inflammation work together to damage epithelial homeostasis and disrupt intestinal immune system function. The connected biological pathways show that patients with dysbiosis or intestinal barrier damage will experience adverse effects when taking high doses of probiotics for extended periods (16–18). The development of small intestine bacterial overgrowth (SIBO) occurs through dysbiosis causing bacterial growth that produces fermentation acids that damage epithelial tissue and lead to mucosal erosion and inflammation (19). The research data showed degenerative villus patterns and coalescence, which match the histological changes that occur in chronic enteropathies and experimental inflammatory gut models (20,21).

The morphometric analysis supported histological findings because it showed that probiotic treatment resulted in shorter villus height and smaller villus area and reduced villus-to-crypt ratio and thinner muscularis thickness. The intestinal absorptive capacity and nutrient uptake efficiency depend on these specific parameters. Their reduction shows that the mucosal absorption system and barrier function have experienced damage. The combination of villus atrophy and muscularis thinning occurs because of prolonged oxidative stress which damages epithelial cells and disrupts their normal renewal process according to previous studies on chronic intestinal stress (22,23).

The research provides new knowledge about non-rodent models because rabbits have a sophisticated hindgut fermentation system which responds strongly to changes in diet and microbial populations. Thus, this study is among the few to demonstrate deleterious duodenal remodelling associated with long-term *L. rhamnosus* administration in a rabbit model, extending the current understanding of probiotic safety beyond rodents (24,25).

The research findings show that translational medicine needs particular probiotic dosing protocols that should be tailored according to both the probiotic strain and the host species. The use of antibiotics for extended periods creates an imbalance in gut bacteria while damaging the intestinal tissue structure. Future research needs to use molecular techniques to study probiotic effects on intestinal tissue by measuring tight-junction proteins and cytokines and oxidative stress indicators. The research needs recovery-phase studies to verify that the documented histological and morphometric changes will fade away after participants stop taking the supplement.

The research study contains one essential limitation which needs to be acknowledged. The study depended on *Lactobacillus rhamnosus* as its probiotic strain at a set dose which restricted its applicability to different probiotic strains and treatment protocols. The research used rabbits as test subjects but this approach might not accurately show how human beings would react to treatment interventions. The study has two main limitations because it only examines histological and morphometric changes while ignoring molecular and biochemical assessments. Lastly the absence of a recovery group leaves uncertainty about whether the observed changes are reversible once the treatment ceases.

In conclusion, ultimately although probiotics show potential in maintaining gut health it is essential to monitor their prolonged use carefully. The negative impact on the lining of the intestine in rabbits as seen in this study highlights the importance of adopting specific and well-founded strategies when contemplating extended probiotic treatment, in both animal and human settings.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

AUTHORS' CONTRIBUTION

Taghreed Al-Fakje designed and supervised the study, performed the experimental work, and contributed to data interpretation. Faeha Al-Mashhadane assisted in histological processing, statistical analysis, and manuscript preparation. Asmaa Al-Nuaimy participated in data collection, literature review, and manuscript editing. All authors read and approved the final version of the manuscript.

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