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The Benefits of β-Glucan Injection during Suckling: Enhancing Immune Responses and Intestinal Health in Newly Weaned Rabbits

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Abstract

The rabbits that have just been weaning are vulnerable to erratic infectious agents, which frequently cause diarrhoea. Natural immunity can also be educated, according to recent research. A quicker and more potent immune response to both homolog and heterogeneous recurrence is a crucial component of acquired immunity. As a result, the current study examined the process of diarrhoea protection provided by intraperitoneal administration of Beta-glucan during the nursing period in weaned rabbits. Sixty-four nursing rabbits were randomly divided into the control group (C) and the trained group (T), according to bodyweight and breed. Physiological saline solution (PSS) and Beta-glucan dissolved in PSS were intraperitoneally administered into the rabbits in the two groups at 6 and 4 days prior to weaning, respectively. Two weeks after weaning, 1 5 rabbits from every group were selected at random for sampling. The trained group's crypt depth and diarrhoea rate were shown to be lower (P 0.04 or P 0.02) in the results. Tumour necrosis factor-alpha (TNF-alpha) and interleukin-15 (IL-10) gene expression increased while interleukin-65(IL-5) gene expression decreased in rabbits receiving Beta-glucan during suckling when compared to the control group (P 0.01). Following weaning, pre-stimulation with Beta-glucan also enhanced the content of ileal secretory immunoglobulin (sIgA) and lysozyme (LYZ) as well as serum immunoglobin A (IgA) and G (IgG) (P 0.04). Additionally, the trained rabbits displayed decreased serum levels of hydroxyl radicals and malondialdehyde (MDA) (P 0.002). Notably, the trained group's ileum mucus' microbial populations had more homogeneity and aggregation. Our findings provided experimental and theoretical support for the use of Beta-glucan-induced trained immunity in preventing intestinal infection after weaning in rabbits. Pre-stimulation with Beta-glucan before weaning improved intestinal health by enhancing the immunity ability of both innate and adaptive immune in the newly weaned rabbits.

Keywords: Beta-glucans, Weaned Rabbits, genes, DNA, vaccination.

Introduction

In recently weaning rabbits, diarrhea is a significant problem because it accounts for 70% of all rabbit infections and frequently causes disturbed intestinal flora and high death rates. The unpredictable nature and diversity of the bacteria-causing illnesses make it difficult to prevent diarrhea in these rabbits. Although recent research has shown that inherent defenses caused by various microbes can also show memory-like behavior, it was once thought that innate defense was nonspecific and nonmemorized in its reaction to infections [1]. In particular,

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early contact with specific antigens and pathogens can prepare the host for a quicker and more potent immune innate response when exposed to subsequent instances of the same or different infections. The term "trained immunity" refers to this immune system recall phenomenon. To encourage the development of natural immunity, immune stimulants like Beta-glucans are widely used. Most fungi's cell walls are made of Beta-glucans, structurally extremely conserved glucose polymers [2]. Sensors for recognizing connections on the outer layer of innate immune cells can identify Beta-glucans as a microbe-associated molecular layout, which triggers a resistant and antimicrobial agent's reaction.

Additionally, there is growing proof that innate immune cells exposed to Beta-glucans have stronger defenses versus encounters with unrelated infections in the future, with improved antibacterial and inflammatory capabilities [3]. According to [4], intraperitoneal vaccination of Beta-glucans in zebrafish, 5 or 1 regular before a pathogen exposure induced a trained innate immune response. The ability of Beta-glucans-induced acquired immunity during nursing to reduce post-weaning diarrhea in rabbits has not, however, been studied yet. Injections of Beta-glucans were given to rabbits six and four days before weaning to improve their immune system's capacity to cope with the transition strain [4]. Following an examination of the mechanism of protection using the evaluation levels of multiple antimicrobial agents and inflamed-connected genes [5]. Study [6] affected supplementing with glutamate (Gln), arginine (Arg), or both on the health of the trash, growth of the gut, and immune system. The does (female rabbits) and their litters were fed various diets as part of the study's methodology. Although the precise procedures utilized for these evaluations were not specified, they included methods like bacterial culture, histological examination, enzymatic assays, and flow cytometry to determine immunity and intestinal growth. Insights into the impact of Gln, Arg, or combination supplements on litter efficiency.

A study [7] found out what happened when rabbits weaned at 20-days of age were fed fish oil (FO), which is high in long-chain, monounsaturated fatty-acids (n-3), instead of lard. There were 24 litters in the trial, 22 of which were placed in the FO group (which received fish oil) and 12 of which were placed in the CG (which received lard). The sole difference between the two experimental diets' feed components—is 0.70% lard (control) or 2.8% Optomega-30 (FO), a dietary supplementation made from salmon oil and containing 60% of ether extract, a mineral-based vehicle. Finally, adding fish oil to the death of recently weaned rabbits produced many advantageous outcomes. Lowering the n-4/n-2 ratios enhanced the fatty acids composition of rabbit meat and fats. The current study [8] examined the impact of Artemisia argyi supplementation on rabbits' production capacity and intestinal barrier. According to the findings of this study, rabbits' ADG, ADFI, and FCR did not change when their diets were supplemented with A. argyi rather than peanut vine and wheat bran. Results of the research demonstrated that, regardless of the inclusion stage, adding A. argyi to the diet had no discernible impact on the rabbits' food intake or rate of gaining weight.

Study [9] determined how different dosages of prickly pear peels (PPP) by-products, a novel ingredient, affected the productivity, digestibility, components of the blood, immune

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response, action of specific digestive enzymes, and economic efficiency of rabbits. There are no known negative health effects from feeding up to 30% prickly pear peels to growing rabbits. The dietary PPP will have a beneficial impact on wellness, with the primary goal of enhancing the immune status in rabbits. It will also improve growth outcomes, digestibility and consuming value, cecum traits, carcass characteristics, some blood constituents, the activity of specific digestive enzymes in pancreatic tissue, intestinal contents, and economic efficiency. The research results showed that the PPP groups had a large impact on the bunnies' ultimate living weight, overall weight growth, and performance indices. Intestinal microbiota and immune system reaction of hens developing necrotic enteritis (NE) were affected by the supplement of Clostridium butyricum [10]. NE is a severe issue for the chicken industry and results in large monetary losses. Three stages made up the experimental layout: a basal diet, a high fishmeal-supplementation diet, and difficulties with Clostridium perfringens. The chickens were split into groups from 1 to 25 days, with one group receiving dietary supplements of C. butyricum and the other not. All teams were given a basal diet for the following 13 days, then from days 14 to 24, received a basal diet with a 50% fishmeal supplement. C. The results showed that the C. perfringens challenge-induced rise in IL-15A gene expression and the decrease in Claudin-1 gene production were suppressed by adding C. butyricum.

Study [11] determined the impact of the astragalus polysaccharides (Aps) and ginseng polysaccharides (Gps) on development, immunological function, liver function, intestinal barrier, and TLR4 signaling systems. 180 weaned piglets were used in the study, which lasted 28 days. They were randomly assigned to one of three therapy teams. Lipopolysaccharide difficulties raised blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), interleukin-1 (IL-1). Study [12] examined the outcomes of feeding weanling rabbit of the New Zealand White (NZW) and Animal Production Research Institute (APRI) breeds beta-glucan (pharmaceutical grade 15%) on growth and carcass quality were investigated. Ingestion of beta-glucan at vaccination doses of 0.15, 0.3 ml per liter of water consumed, particularly the dose of 0.3 ml liter in both rabbit breeds, the faster body weight growth (BWG), lower feed conversion ratio (FCR), and lower overall feed consumption (FC). Every element of developing rate varies noticeably as a result of breed effect. The beta-glucan and breeding combination impact significantly enhanced the BWG, FC, and FCR. As a result, giving rabbits a vaccination of 0.10 or 0.3 ml of Beta-glucan per liter of water to drink had a positive impact on their ability to grow and their general health.

Study [13] examined the effects of supplements containing Clostridium butyricum (CB) and a bacteriophage cocktail (BP) on a variety of rabbit improvement-related, serum biochemical, intestinal digesting and oxides enzyme, intestine morphological, immunological, and cecal microbiota-related parameters. Rabbits' average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) improved when CB and BP were added to their meals in compared to the CN diet. In the ileum, CB the supplementation boosted the synthesis of digestive enzymes such as trypsin and amylase the jejunum and duodenum saw a significant

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increase in chymotrypsin activity. As a result, rabbits' growth rate, intestinal wellness, antioxidant capacity, immunological activities, and composition of the cecal microbiota were all improved by dietary supplementation with CB and BP. The oregano essential oil affected rabbits' intestinal barrier and production capacity [14]. One hundred ninety-two weaned Hyla rabbits (2 months old) of identical body weight (1268.5 25 g) were assigned at random to a single of four groups (24 reproduces each group, two rabbits per replication) and given one of four different diets: a basal diet (CG), a normal diet containing 0.02% of oregano essential oil (LO group; the innocence of the essential oil of oregano was 5%), a basal diet containing 23-days testing followed by 7-days of adaptation in the course of the experiment. As a result, oregano essential oil as a dietary supplement modifies immunological reactions and strengthens the intestinal wall. Additionally, convincing evidence that using essential oregano oil rather than antibiotics can improve rabbit health.

Mechanism and impact of an intraperitoneal vaccination of Beta-glucan during the nursing phase on preventing diarrhea in newly weaned rabbits were investigated [15]. 64 suckling rabbits were separated into both groups at random constructed on weight and litter: the CG (C) and the TG (T). The rabbits in the two groups were given intrauterine injections of physiologically saline-solution (PS) and Beta-glucan dissolved in PS, accordingly, 6 to 4 days before weaning. Ten rabbits from Random selection were made for each group. An illustration after two weeks of weaning. According to the outcomes, the trained group had less diarrhea and a shallower crypt in the ileum than the CG. In comparison to the CG, the rabbits that received Beta-glucan throughout the nursing period showed elevated gene expression of tumor necrosis factor-alpha (TNF-) and interleukin-10 (IL-11) and reduced gene expression of interleukin-5 (IL-5) respectively. Aiming to provide a fresh exploration and treatment basis of effective oversight of diarrhea in recently weaned rabbits, our study examined immunoglobulin heights and fundamental organization of the digestive tract microbiota in newly-weaned rabbits based on this assumption.

Materials and Methods

Statistic evaluations

The data on growth results, blood, and intestinal indicators in weaning rabbits was analysed using the t-test. At the same time, Chi-square test was used to analyze the data on diarrhea rate. The non-parametric Test of a Wilcox rank-sum was used to evaluate diversity indexes (Shannon) and the proportions of species-genera between both groups to examine microbiological data. The principle coordinating analysis (PCoA) distance matrix and the ANOSIM- analysis based on the UniFrac distance without weights were used for comparing the beta range of the community of bacteria LEfSe and was performed utilizing a web-based application.

rRNA gene sequencing and bioinformatics analysis

The microbiological genomic DNA was obtained from the ileal mucus (iMs) tissues using a pounding beads and phenol-chloroform technique, and the results were then allowed to thaw

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at four °C. DNA was purified and measured for content water (1.5ng/mL) with (TRIS-EDTA) solution. Using barcoded primers (515F-806R), water DNA was utilized to amplify the V4- hypervariable areas in the 14S rRNA gene amplicons. Following the manufacturer's recommendations, the amplifiers were taken from 5% agarose gels and purified with the AxyPrep DNA Gel Extraction Rabbit. The Fluorometer from Experimental Thermo and the Agilent Bioanalyzer 2200 system assessed the library's quality. Fast 10 technologies were used to do excellent filtering on the raw tag to produce Clean Tags of the highest caliber. Using the UPARSE program, all effective tag sequences were grouped into operational taxonomic units (OTUs) based on 98% identity criteria.

Hematological and ileac mucus indicators analysis

ELISA rabbits were used to quantify the levels of IG-G and IG-A in the serum (Angle Gene Biotechnology, Nanjing, China). I was using ELISA rabbit, the manufacturer's directions for using quantified secretory immunoglobulin A (sIgA) in the mucous of the ileum.

The serum's degree of oxidative stress

Using commercialised (ELISA) rabbits from Angle Gene Biology, we assessed malonaldehyde MDA, total antioxidant capacity T-AOC, and hydroxyl radicals in the bloodstream in accordance with the manufacturer's instructions.

Results

Analyses using quantitative real-time PCR (qRT-PCR)

The reagent TRIzol was used to extract the total RNA from the ileum and append tissues. A genetic acid/protein analysis and agarose gel electrophoresis was used, accordingly, to measure RNA concentration and structure. Script cDNA Synthesis Rabbit was used to conduct reverse transcription operations. On a LightCycler® 450 Instrument II, qRT-PCR was carried out using Power SYBRTM Green PCR Master Mix. The 2CT approach was used to normalize the connected mRNA reaction of the target genes to GAPDH. Table S2 contains primer combinations.

Intestinal morphology

The paraffin-embedded, 6-μm thick portions of the fixed intestine segment were removed after they had been dehydrated. After deparaffinization and rehydration, the parts were stained with hematoxylin and eosin and allowed to dry. Eight portions of every rabbit, each with at least five photos, were collected. They were utilizing an Olympus CK 40 microscope. The villus length and crypt depth were measured, and the villi-crypt ratio was calculated by the technique developed.

Growth performance and Fecal score

The feed conversion ratio (FCR), average daily feed intake (ADFI), and average daily gain (ADG) were among the variables that had to be calculated for the study. These variables were calculated using data from the rabbits' feed consumption and body weight measurements

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taken on the 40th (1 week after weaning) and 50th (2 weeks after weaning). Every morning during the same times, the rabbits' diarrhea was observed, and their fecal quality was graded using for 0 to 3. Regular bean-shaped feces obtained a rating of 0, pasty feces obtained a rating of 1, semiliquid feces obtained a rating of 2, and liquid wastes received a value of 3. Rabbits were deemed to have diarrhea if their fecal score was less than 1. The overall incidence of diarrhoea was computed as formerly mentioned, and the regular day's diarrhea rate for each category was identified: diarrhea pace (%) =150 (rabbits overall and the number of rabbits who have diarrhoea).

Sample collection

Ten rabbits from both groups were selected at random at 55-day testing. The ear margins veins were used to collect blood samples, which were then centrifuged at three °C to obtain serum. All rabbits were humanely killed after blood was collected using a blinded approach, per the instructions. On the ice, the ileum and appendices were gathered for subsequent extracting of RNA. The ileum was then sheared, cleaned with clean microscopy slides, and rinsed with phosphate-buffered saline (PBS), which is ice cold. To preserve tissue and mucus samples for later investigation, liquid nitrogen was used to fast freeze them. Additionally, a different section of the ileum from each rabbit, spanning about 1-2 cm in length, was collected and preserved was 3% formalin buffered saline solution before being subjected to histologic analyses.

Accommodation, animals, and design of experiments

Both the CG (n = 30) and the trained group (n = 30) were made up of the same number of 61 healthy New Zealand-born rabbits (age 20 days) all of identical gender and weight. A vaccination of Beta-glucans solutions (0.04 g/ml, 63 mg/kg body weight) was administered intraperitoneally to rabbits in the conditioning group at 26-days old (1 week before weaning) and 1 month old (under 4days before weaning), respectively (Figure (1)). The CG got the same dosage of intraperitoneal injections of sterile water instead of the yeast-derived Beta-glucans. On the day of weaning (1 month old), the bunnies from both groups were moved from their does to a typical establishing, where they were kept in a controlled setting with lights alternated cycles of 13 h light and 6 h darkness. The rabbit was raised independently throughout the trial in their cage and received commercially produced food twice per day between the hours of 8 am and 5 pm. Additional details on the particular components and biological breakdown of the food applied the research are supplied in Table (1).

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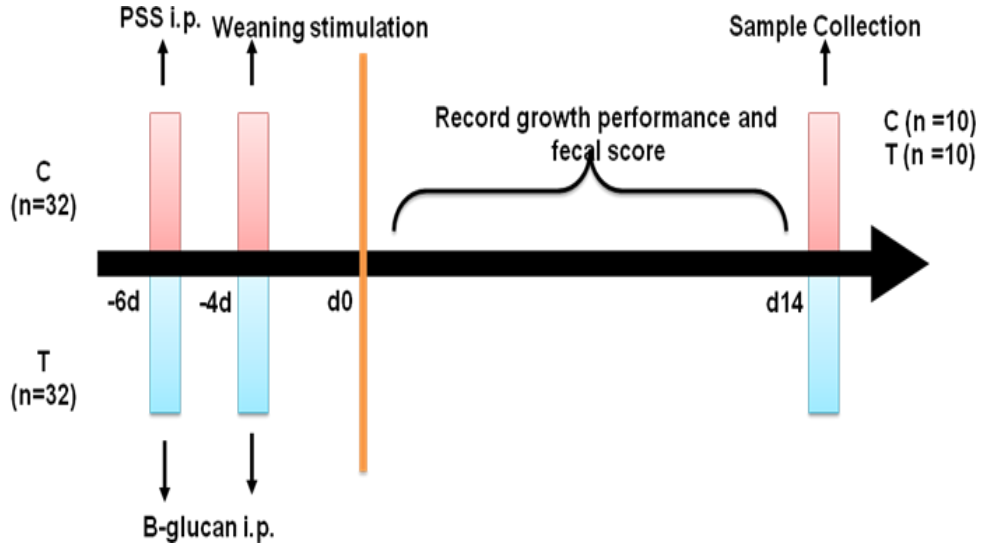

Figure (1): physiologic saline solution (PSS). CG, TG

Result

Performance in Production in Rabbits

Pre-stimulation with Beta-glucan not affected the (ADG1, ADFI1, FCR1) compared to the C (Table (1)). Still, it did reduce the rate of diarrhea within each 7 days after weaning (f = 0.030) and tended to do so during the next both weeks (f = 0.060) (Table (2)). Among the Beta-glucan trained group and a control team. There were no differences in the organ-specific index of the liver, thymus, spleen, sacculus-rotundus, and section (Table (3)).

Table (1): Impact of Beta-glucan stimulating on rabbit development efficiency (*n* = 30 rabbit/group)

Items		P-value
ADFI, g/d	First week	0.242
	Second week	0.368
	Whole Two weeks	0.348
ADG, g/d	First week	0.525
	Second week	0.184
	Whole Two weeks	0.614
FCR	First week	0.137
	Second week	0.344
	Whole Two weeks	0.978

Table (2): Impact of Beta-glucan stimulating on rabbits' rate of diarrhea (n = 30 rabbit/group)

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Items	P-value
First week	**0.037**
Second week	**0.416**
Whole Two weeks	**0.078**

Table (3): Impact of Beta-glucan stimulating on the Rabbit organ indices (*n* = 20 rabbit/group)

Items	P-value
Appendix	**0.707**
Sacculus rotundus	**0.328**
Spleen	**0.738**
Thymus	**0.669**
Liver	**0.624**

Morphology of the ileum histologically

The ileal villus edge of the TG had a usual form, whereas the CG was significantly exfoliated and degenerated (Figure (2)).

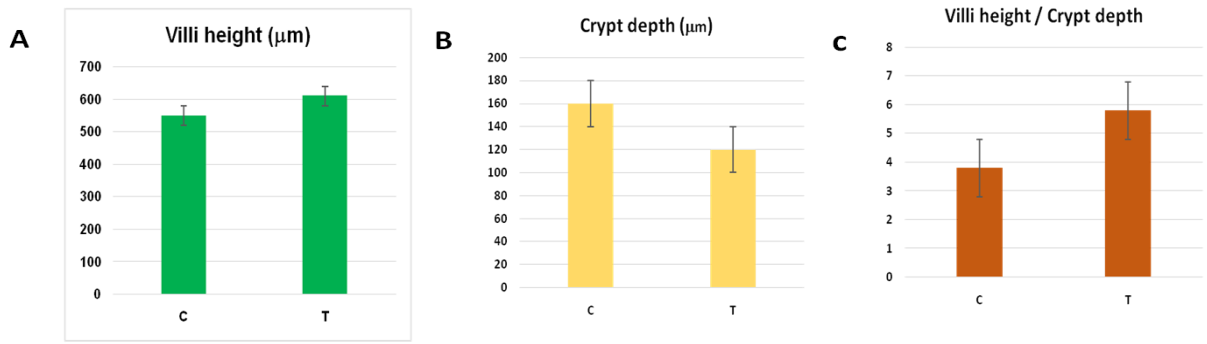

Figure (2): The ileum's histological examination in newborn rabbits

TLR expression, LYZ, and Muc2 stages, and signs of inflammation have been observed in the ileum of newly weaned rabbits

To investigate the variation in chronic inflammation between the two groups, the related levels of IL-5, IL-8, and IFN-TNF in the ileum and appendices was measured. Pre-injection of Beta-glucan considerably enhanced the expression related to TNF-IL-8 in the appendix (f =0.01) while dramatically decreasing the relative expression of IL-5 (f 0.04), as seen in (Figure (3)). (IFN-TLR 2,3,4) expression levels in annexed. Those of (TNF, IFN, and IL-8, IL-5) in the ileum were unaffected by prestimulation with Beta-glucans (f > 0.04), although TLR2 expression in the rabbit ileum was somewhat reduced (f = 0.074) (Figures (3) and (4)). The level of LYZ in iMs was considerably increased in rabbits from the training group (f

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0.002), and it also tended to grow in the appendix (f = 0.044). Furthermore, rabbits stimulated with Beta-glucan increased Muc2 content in their ileum (f = 0.055) (Figure (5)).

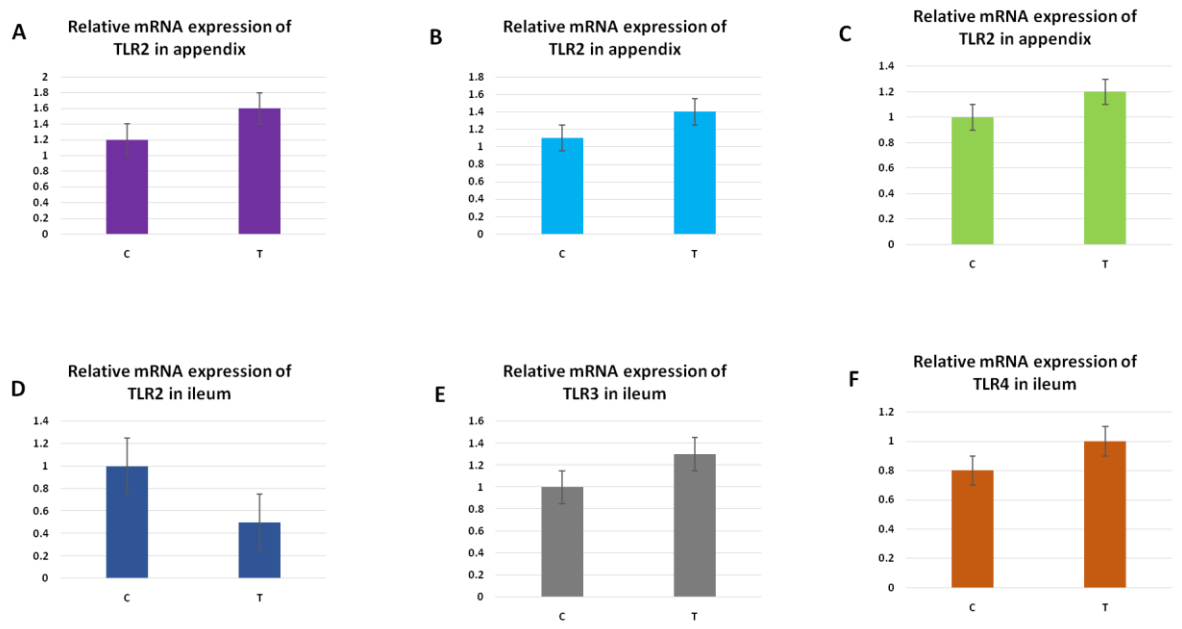

Figure (3): Showing of the genes intestinal TLRs in young rabbits

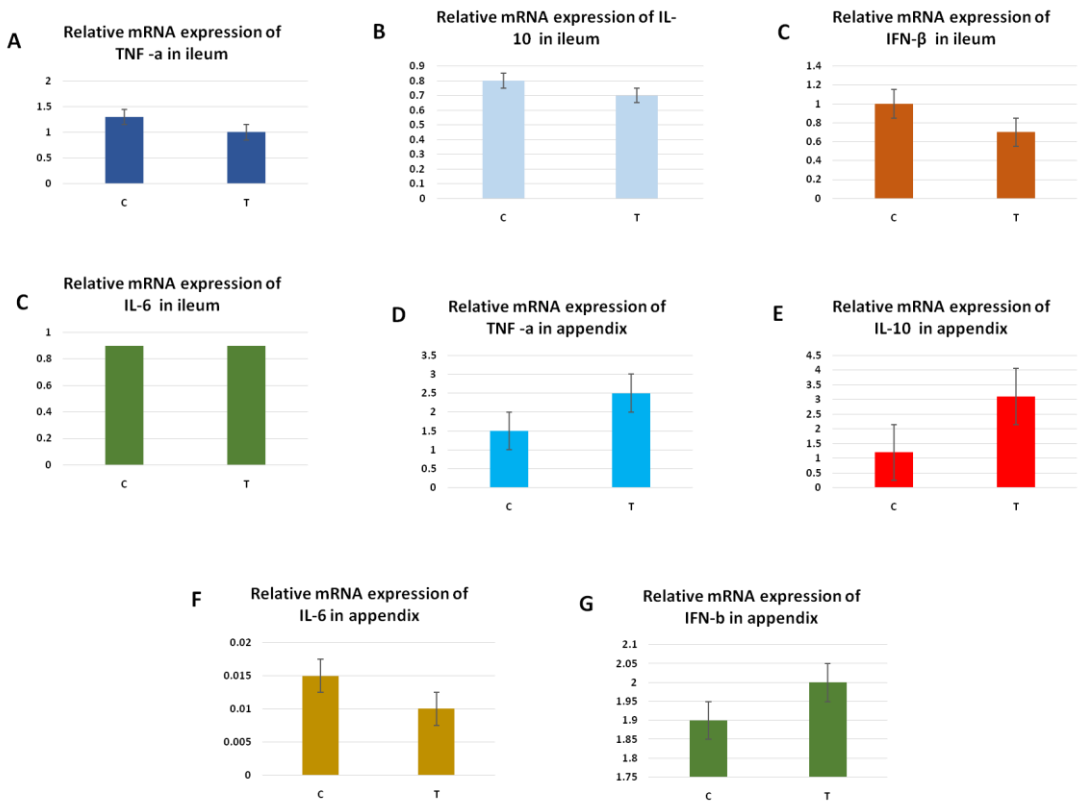

Figure (4): Showing of the genes intestinal inflammatory cytokine in young rabbit

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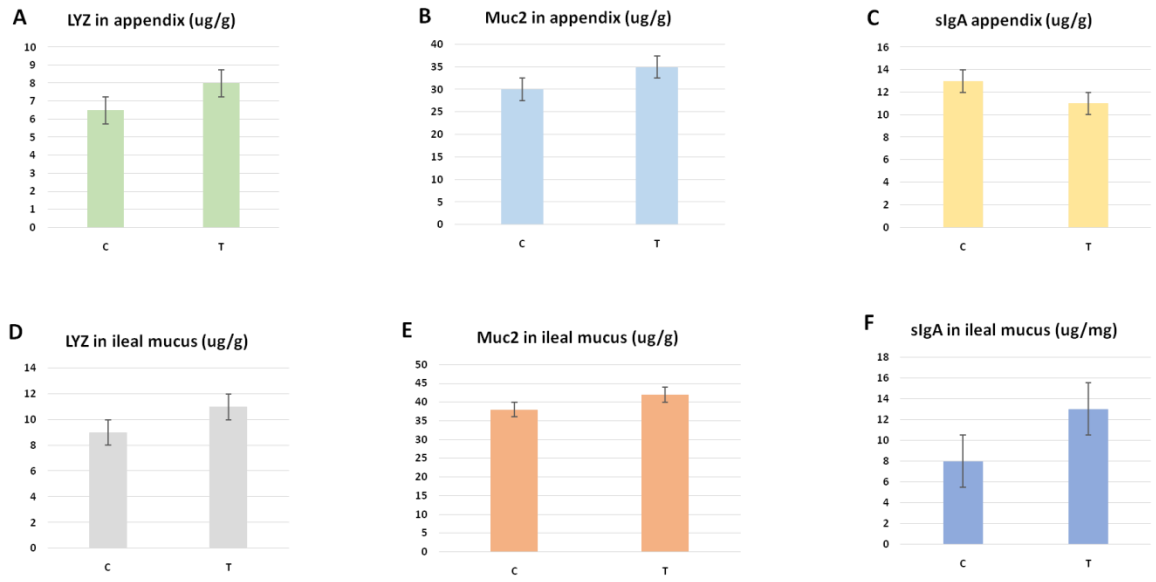

Figure (5): Young rabbits' levels of Muc2 and intestinal lysozyme

The levels of ileal mucus sIgA and serum immunoglobulins in weaned rabbits

It was measured how much IgA-IgG was present in the serum 15 days to the day after weaning. Following weaning, the trained rabbits secreted more IgA and IgG, Additionally, the trained rabbits had increased sIgA concentrations in iMs (P = 0.002), but pre-stimulation did not affect sIgA concentrations in the section (Figure (6)).

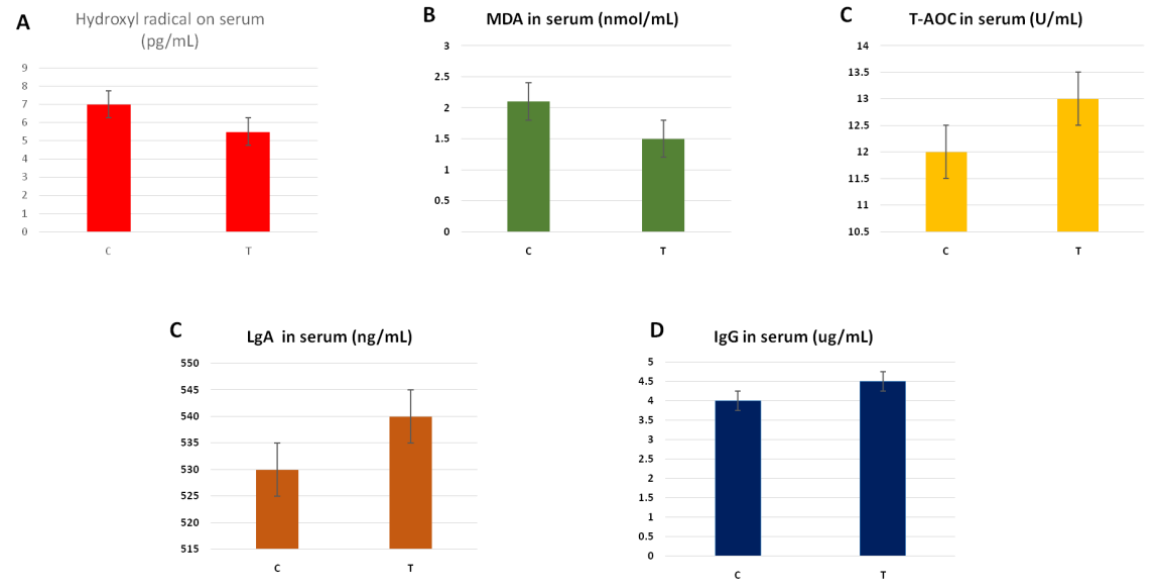

Figure (6): The immunoglobulin levels and serum antioxidant status of young rabbits

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http://www.veterinaria.org
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Level of antioxidants in serum

The weaned rabbit's level of oxidative stress was also found. According to prestimulation with Beta-glucan decreased serum MDA and hydroxyl radical groups (f = 0.003 and f 0.001, respectively) but did not affect T-AOC levels (f = 0.723).

Conclusion

This research confirmed that enhancing innate defenses through injection of Beta-glucan before weaning could be a potential preventative measure for reducing the rate of diarrhea in rabbits following weaning. Further research revealed that weaning anxiety was reduced and intestinal structure was improved in rabbits that had been weaned by an immunologic procedure that honed an immune system's adapted and innate responses. It was the initial of this type to investigate the impact of preweaning Beta-glucans vaccination on hindering “post-weaning” diarrhea in rabbits, unveiling a novel technique for safeguarding recently weaning animals, such as piglets, calves, and sheep, against unpredictable complex microorganisms.

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