

Effect of Dexamethasone in Feed on Intestinal Permeability, Differential White Blood Cell and Immune Organs in Rabbits

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ABSTRACT

An experiment was conducted to evaluate the effect of stress on intestinal permeability induced by administration of dexamethasone in feed. Male albino rabbits (n=10) was purchased from local market of Hyderabad and brought to Animal House, FAHVS SAU Tandojam. Rabbits were assigned to the two groups; Group A (control) and group B (dexamethasone fed). Rabbits were kept in a monitored facility with full access to food and water that was age-appropriate. For the rest of the trial, the DEXF treatment group was switched to feed containing DEXF1X (0.57 ppm) on day 3. The results revealed that dexamethasone-fed rabbits (Group B) exhibited a significant decrease in body weight (2104 ± 28.66 g) compared to the control group (Group A) (2506 ± 45.81 g). The serum fluorescein Isothiocyanate dextrin level in group -B was significantly ($P < 0.05$) Higher (0.29 ± 0.3 kg/ml) than that of rabbits in group A (control) with mean serum FTTC-D (0.22 ± 0.01 kg/ml) Total liver aerobic bacteria in group -B supplemented dexamethasone fed was significantly ($P < 0.05$) Higher ($1.2 \pm 0.45 \log_{10}$ Cfu/g) than that of rabbits in group -A (control) with mean total liver aerobic bacteria ($0.13 \pm 0.05 \log_{10}$ cfu/g). The WBC count was significantly higher in Group B ($9.74 \pm 1.22 \times 10^3/\mu\text{L}$) than in Group A ($7.02 \pm 0.48 \times 10^3/\mu\text{L}$). Heterophils count was significantly elevated in Group B ($40.44 \pm 7.42\%$) compared to Group A ($25.08 \pm 5.32\%$). Lymphocytes count was significantly lower in Group B ($46.12 \pm 5.32\%$) compared to Group A ($58.12 \pm 4.67\%$). Monocytes count showed no significant difference between the groups, with Group A ($8.26 \pm 1.47\%$) being slightly higher than Group B ($6.96 \pm 1.83\%$). Eosinophils count was significantly lower in Group B ($3.70 \pm 1.22\%$) compared to Group A ($5.72 \pm 1.07\%$). Basophils count showed no significant difference between the groups, with Group A ($2.84 \pm 0.57\%$) being slightly higher than Group B ($2.68 \pm 0.30\%$). The H/L ratio was significantly

higher in Group B (0.87 ± 0.02) than in Group A (0.43 ± 0.01). Effect of dexamethasone on lymph nodes, thymus, spleen and Intestine Morphology was observed through histological study. Thymic lobule length was significantly lower in Group B (380 ± 2.30 μm) compared to Group A (520 ± 4.97 μm). Thymic lobule width was significantly lower in Group B (180 ± 1.73 μm) compared to Group A (240 ± 2.66 μm). White pulp length was significantly lower in Group B (340 ± 6.14 μm) compared to Group A (470 ± 7.27 μm). White pulp width was significantly lower in Group B (140 ± 1.74 μm) compared to Group A (220 ± 3.82 μm). These findings indicate that dexamethasone administration in the feed of rabbits leads to a decrease in body weight, altered WBC count, increased heterophils count, decreased lymphocytes count, reduced eosinophils count, and an elevated H/L ratio. Additionally, dexamethasone adversely affects thymic lobule length and width, as well as white pulp length and width in the thymus and spleen, respectively.

Keywords: Blood profile, Spleen histology, Dexamethazone, Thymus, Body weight, Rabbit

INTRODUCTION

In animal models of opportunistic infections, bone pathologies, and nutrition transport, dexamethasone (Dex), a synthetic GC analogue with recognised anti-inflammatory and cell-mediated immunosuppressive properties, has been utilised to imitate stress conditions. A small number of studies have looked at the direct effects of Dex on enteric permeability in chickens, and their findings generally support those from mouse and human models that show prolonged GC exposure causes immunosuppression. These studies have found decreased defences against luminal bacteria, increased heterophil: lymphocyte ratios, decreased secondary lymphoid tissue weight, and barrier dysfunction as indicated by increased recovery of the marker molecule fluorescein isothiocyanate dextran (FITC-d). Stress has also been shown to cause changes in enteric pathogen virulence phenotype, shifts in intestinal microbial communities towards a state of reduced diversity and richness, and systemic bacterial infection (Bailey et al., 2010) [1]. However, nothing is known about how Dex, a disruptor of intestinal integrity, interacts with the potential function of the prevailing microbial community. Insights for therapeutic targets against stress-mediated enteric complications in broilers could be gained from describing the role of resident intestinal microbes in a chronic exogenous GC-mediated stress response. This information could also help define the models in which Dex would be an effective comparative treatment or risk factor.

Dexamethasone (DEX), a synthetic glucocorticoid derivative, is used mostly for therapeutic purposes due to its ability to reduce inflammation and inhibit the immune system (Watteyn et al., 2013) [2]. In the poultry industries nowadays, stress is one of the main risk factors for chicken illnesses. According to Shini et al. (2010) [3], DEX, an immunosuppressant medication, is often used to research the role of stressors in the onset and progression of poultry illnesses. DEX was predicted to have effects akin to high corticosterone concentrations and to activate stress-related signalling pathways since it is near to natural corticosteroids (Calefi et al., 2016) [4]. Less noticeable were the effects of DEX on lymphocytes from the popliteal, mesenteric, and spleen (Jeklova et al., 2008) [5]. Aengwanich (2007) [6] studied the effects of DEX therapy, which included granulocytophilia and decreased mononuclear cells in humans, lymphopenia and neutrophilia in rats, neutrophilia and eosinopenia linked with lymphopenia, monocytosis in cattle, and lymphopenia and neutrophilia in pigs. In broiler chicks treated with DEX, immune organ weight loss (Vicuna et al., 2015) [7] and reduced blood lymphocyte levels (Aengwanich, 2007; Afrose et al., 2018) [6,8] were also observed. Along with liver enlargement, corticosteroid therapy in birds was shown to reduce the weight and function of lymphoid organs such as the thymus, bursa of Fabricius, and spleen (Mehaisen et al., 2017) [9].

The intestinal mucosa is made up of a single layer of epithelial cells that performs a variety of tasks, such as secreting mucus and enzymes, absorbing nutrients, and acting as a barrier between the external and internal environments. Maintaining epithelial integrity is therefore crucial for raising healthy, feed-efficient poultry from the perspectives of nutrition and disease. Multiple elements, including enzymes, pathogenic agents, toxins, and hormones, have an impact on barrier function (Soderholm & Perdue, 2001; Lamprecht & Frauwallner, 2012) [10,11]. The distribution and integrity of tight junctions play a major role in regulating the continuity of the epithelium, which is crucial for regulating both paracellular and transcellular permeability. Induced alterations in intestinal permeability linked to a transient displacement of the tight junction have been seen in animal models of acute and chronic stress (Maejima et al., 1984; Matter & Balda, 2007) [12,13]. Other enteric changes linked to stress include altered ion, fluid, and mucus output as well as changes to gut motility and permeability (Alverdy & Aoy, 1991; Karavolos et al., 2008) [14,15]. Mast cells, which play a key role in the brain-gut axis and translate stress signals into the release of a variety of neurotransmitters and pro-inflammatory cytokines, have

been linked to these changes (Groschwitz & Hogan, 2009; Bailey et al., 2011; Lamprecht & Frauwallner, 2012) [11,16,17]. These cytokines have a significant impact on gastrointestinal physiology.

Dexamethasone has been found to affect the integrity of enteric tight junctions in breast tissue and at the blood-brain barrier, but there are few data on these tight junctions in the gastrointestinal tract (Boivin et al., 2007; Tenenbaum et al., 2008) [18,19]. However, it is also often used to cause opportunistic infections by allowing bacteria from the digestive system to enter the bloodstream (McGruder et al., 1995; Huff et al., 1999; Wideman and Pevzner, 2012) [20-22]. Although research utilising dexamethasone for the induction of skeletal disorders with aetiologies partly involving intestinal bacteria have been published (Huff et al., 1998; Wideman & Pevzner, 2012) 23 [22,23], no studies specifically investigating the effects of dexamethasone on enteric permeability have yet been described in poultry. We have previously shown that poorly digested diets and feed restriction may have a negative impact on the intestinal barrier function, leading to an increase in the permeability associated with intestinal inflammation (Tellez et al., 2014; Kuttappan et al., 2015; Vicuna et al., 2015) [7,24,25]. Dietary administration of DEX in rabbits may be a simple, regulated, and adaptable method in the investigation of host stressor adaptation. The proportional amount of change in immunological organ weight caused by dietary DEX treatment has not yet been thoroughly studied.

In view of the stated above statements, the current study is planned to evaluate the alterations in gut permeability under stress induced by administration of dexamethasone in feed in rabbits.

MATERIALS AND METHODS

Experimental Animals and Design

An experiment was conducted to evaluate the effect of stress on intestinal permeability induced by administration of dexamethasone in feed. Male albino rabbits (n=10) was purchased from local market of Hyderabad and brought to Animal House, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. Rabbits were assigned to the two groups; Group A (control) and group B (dexamethasone fed). Rabbits were kept in a monitored facility with full access to food and water that was age-appropriate. For the rest of the trial, the DEX treatment group was switched to feed containing DEXF1X (0.57 ppm) on day 3.

Intestinal Permeability

Serum Determination of FITC-D Leakage

According to (Kuttappan et al., 2015; Vicuna et al., 2015) [7,25], fluorescein isothiocyanate dextran (FITC-D; MW 3-5 kDa; Sigma Aldrich Co., St. Louis, MO) levels were found in serum. Blood was drawn from rabbits, maintained at room temperature for three hours, then separated into serum and red blood cells using a centrifuge (500 X g for 15 minutes), and then diluted 1:1 in PBS. The Synergy HT, Multi-mode microplate reader from BioTek Instruments, Inc., Winooski, VT, was used to measure the concentrations of FITC-D in the blood at 485 nm for excitation and 528 nm for emission. The observed fluorescence was then contrasted with a standard curve containing known FITC-D concentrations. Each rabbit's gut leakage was noted.

Bacterial Translocation

A part of each rabbit's liver was aseptically removed, collected in sterile bags, homogenised, weighed, and diluted 1:4 wt/vol with sterile 0.9% saline to assess bacterial translocation (BT) from the digestive tract to blood circulation. To determine the quantities of total aerobic bacteria translocation, serial dilutions of each sample were produced and plated on tryptic soy agar (TSA, catalogue no. 211822, Becton Dickinson, Sparks, MD).

Blood Collection

Five rabbits from each group were chosen at random to have blood samples taken. The samples were placed in plastic tubes that had been heparinized, and blood plasma was separated by centrifugation at 250 g for 10 min at 4 °C and then refrigerated at 20 °C. Using a 3ml sterile syringe, blood samples were drawn from the heart and put into 1.5ml tubes containing anticoagulant (EDTA). After that, the samples were sent to the Life Diagnostic and Molecular Laboratory in Hyderabad to determine the differential cell counts. The total counts of white blood cells (WBC), heterophils, lymphocytes, monocytes, eosinophils, and basophils were among the hematologic measures of heparin anticoagulated blood. Calculating the heterophil/lymphocyte ratios (H/L), a stress indicator, requires dividing the number of lymphocytes in 1 mL of peripheral blood by the number of heterophils.

White Blood Cell Counts ($\times 10^3/\mu\text{L}$)

A droplet of oil was applied to a dry blood film that was well-

stained, and then it was covered with a clean cover slip. An area that demonstrated good staining and spreading was selected using the x10 objective. Then, the microscope was set to 40X and/or 100X with oil immersion objective lenses. The differential counter was reset to zero prior to initiating the counting process. The counting started by identifying all the white blood cells (WBCs) in a single longitudinal direction, with the button on the differential counter pressed corresponding to each cell type. If the selected area was traversed before reaching a count of 100 cells, the field was shifted one notch upwards (or downwards). Subsequently, the count continued in another longitudinal direction, but this time moving in the reverse direction. The counting process carried on this way until the alarm on the counter sounded. By this point, the total number of WBCs counted should have reached 100. The respective percentages of each type of WBC were then directly recorded from the counter.

Histopathology of Immune Organs

Samples of the immune systems of control and experimental rabbits were obtained in order to study the effects on the bursa cloacalis, spleen, and thymus. As soon as the rabbits were killed, tissue samples were obtained by dissection. The tissue samples were stored in 10% formalin after collection. The samples were then saved for a standard staining technique.

Processing of Tissues for Histological Study

The fixed tissues were dehydrated for histological examinations using a sequence of escalating alcohol grades, followed by three changes in xylene. At intervals of 30 minutes, the tissues were then infused with melted paraffin of varying grades (49 °C, 55 °C, and 58 °C). Following the paraffin (58 °C) embedding of the tissues, slices of the tissues were cut at a thickness of 6 µm using a sliding microtome (MIC 509, Euromex, Japan). Following cutting, the pieces were stretched in a flotation bath of lukewarm water heated to 37 °C, and they were then mounted on clean slides using an adhesive (egg albumins) and dried on a slide warmer at the same temperature. After that, Mayer's Hematoxylin and Eosin (H & E) was used to stain the sections.

Morphometric Study and Biometric Measurement

Light microscopy was used to examine the lymphoid tissues' histological features at low (magnifications of 10) and high (magnifications of 40). Pictures were taken of the chosen samples. Then, using a calibrated stage micrometre in mm (micrometre), the biometric measurements of various histological structures of the lymphoid tissues were carried out. A morphometric analysis of the sections from the control and treatment groups was conducted. For biometric measurement, the thymic lobule of the thymus, the bursal follicle of the bursa cloacalis, and the white pulp of the spleen were all taken into consideration.

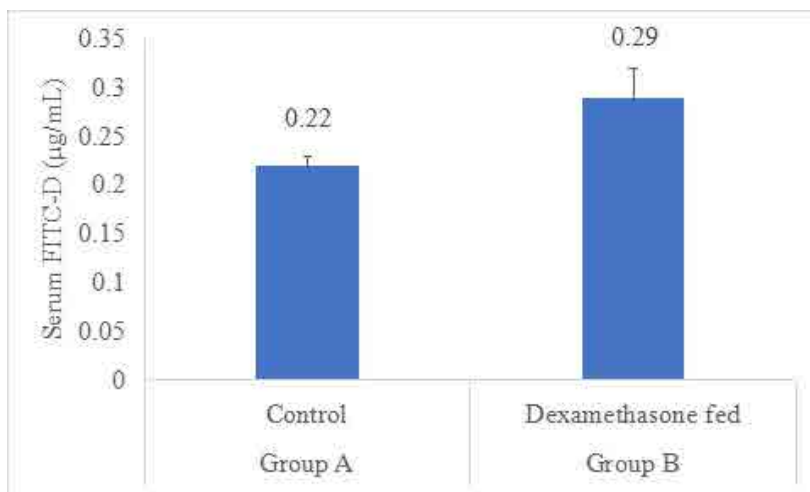
Statistical Analysis

Statistical analysis of the data was performed using statistics 8.1 computer programme (Satatix, 2006). When applicable, the least significant difference (LSD) test was used to compare the differences between the treatments.

RESULTS

Serum Fluorescein Isothiocyanate-dextran Level (FITC-D)

Dexamethasone feed (DEXF) were tested and compared to the control, to allow the dexamethasone administered in the feed to affect the intestinal mucosal leakage as measured by FITC-D (Figure-1). Data indicates that serum FITC-D of rabbits in group B was significantly ($P < 0.05$) higher ($0.29 \pm 0.03 \mu\text{g}/\text{mL}$) than that of rabbits in group A (control) with mean serum FITC-D ($0.22 \pm 0.01 \mu\text{g}/\text{mL}$). Following statistical analysis of the data, it was discovered that group B's serum FITC-D levels were considerably ($P < 0.05$) greater than group A's. By removing one sample that was 2 SD out from the group mean, the Dexamethasone-fed group was shortened. However, treatment-related clinical symptoms, such as lethargy and pallor, and mortality were identified in the B group. The DEXF group was statistically greater than the control, indicating increased paracellular leakage through the gut epithelium linked with disruption of tight connections.



^{a,b}Superscripts among the bars indicates significant difference between them

Figure-1: Serum FITC-D (µg/mL) of rabbits in control and dexamethasone fed groups.

SE± = 0.0983

LSD @ 0.05 = 0.1141

P-Value = 0.0307

Total Liver Aerobic Bacteria (Log10 cfu/g)

A result on the effect of dexamethasone in feed on total liver aerobic bacteria is presented in Figure-2. Data indicates that total liver aerobic bacteria in group B supplemented dexamethasone fed was significantly (P<0.05) higher

(1.26±0.45 log10 cfu/g) than that of rabbits in group A (control) with mean total liver aerobic bacteria (0.13±0.05 Log10 cfu/g). Statistical analysis of the data revealed that total liver aerobic bacteria in group B was significantly (P<0.05) higher than group A.

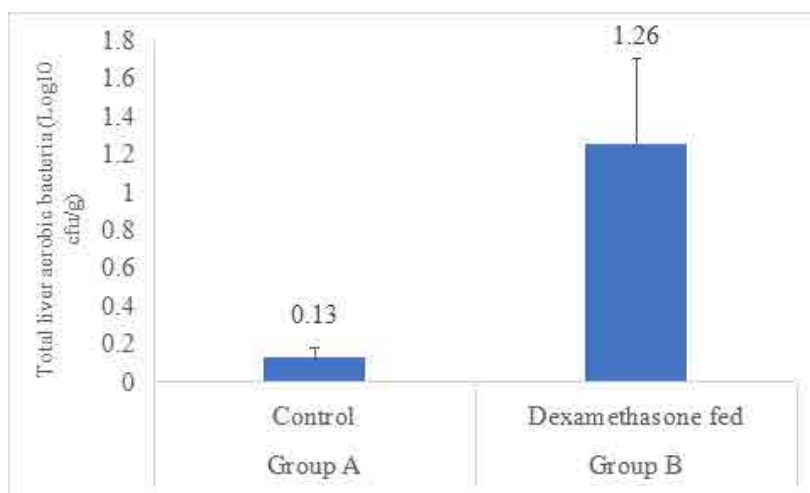


Figure 2: Total liver aerobic bacteria (Log10 cfu/g) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

SE± = 0.8733

LSD @ 0.05 = 0.1914

P-Value = 0.0244

Body Weight (g)

Results on the effect of dexamethasone in feed on body weight of rabbits are presented in Figure-3. Data indicates that body weight of rabbits in group A (control) was significantly

($P < 0.05$) higher ($2506 \pm 45.81g$) than that of rabbits in group B supplemented dexamethasone fed with mean body weight ($2104 \pm 28.66g$). Statistical analysis of the data revealed that body weight of rabbits in control (A group) was significantly ($P < 0.05$) higher than group B.

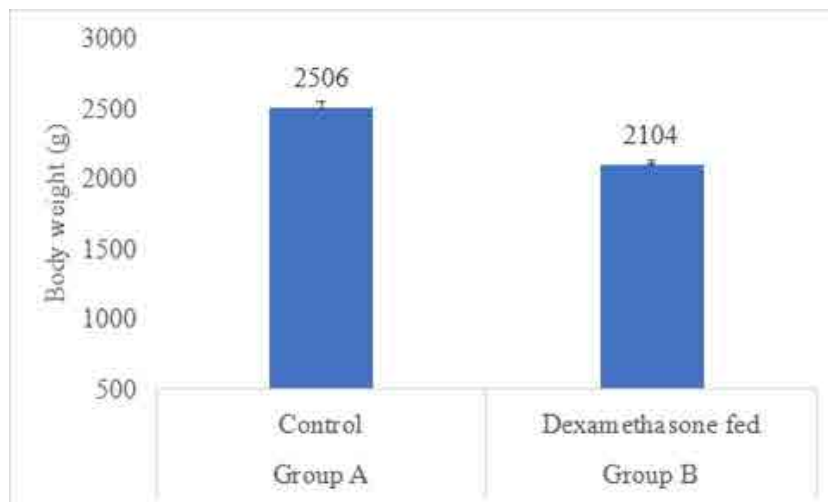


Figure 3: Body weight (g) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE \pm = 18.762$$

$$LSD @ 0.05 = 43.264$$

$$P\text{-Value} = 0.0027$$

WBC Count ($\times 10^3/\mu L$)

Results on the effect of dexamethasone in feed on WBC count of rabbits are presented in Figure 4.4. Data indicates that WBC count of rabbits in group B (dexamethasone fed) was significantly ($P < 0.05$) higher ($9.74 \pm 1.22 \times 10^3/\mu L$) than group

A (control) ($7.02 \pm 0.48 \times 10^3/\mu L$). Statistical analysis of the data revealed that WBC count of rabbits in control (A group) was significantly ($P < 0.05$) lower than group B.

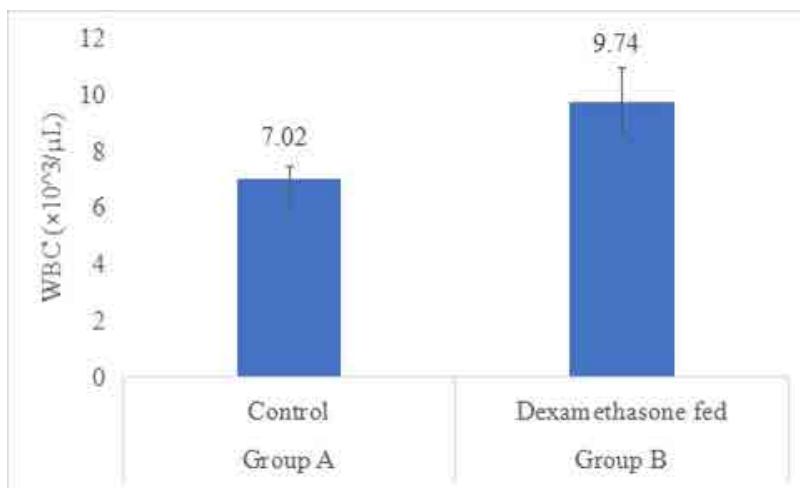


Figure 4: WBC count ($\times 10^3/\mu\text{L}$) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE\pm = 0.1732$$

$$LSD @ 0.05 = 0.3994$$

$$P\text{-Value} = 0.0011$$

Heterophils count (%)

Results on the effect of dexamethasone in feed on heterophils count of rabbits are presented in Figures 4,5. Data indicates that heterophils count of rabbits in group B (dexamethasone

fed) was significantly ($P < 0.05$) higher ($40.44 \pm 7.42\%$) than group A (control) ($25.08 \pm 5.32\%$). Statistical analysis of the data revealed that heterophils count of rabbits in control (A group) was significantly ($P < 0.05$) lower than group B.

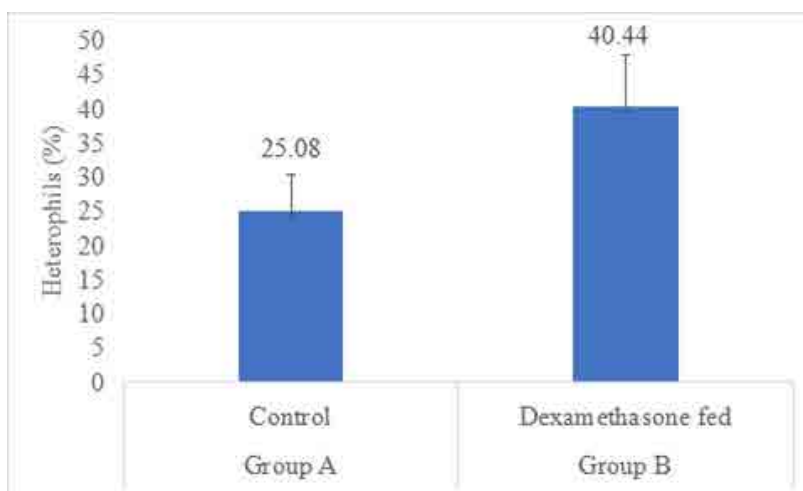


Figure 5: Heterophils count (%) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE\pm = 0.6964$$

$$LSD @ 0.05 = 1.6059$$

$$P\text{-Value} = 0.0017$$

Lymphocytes Count (%)

Results on the effect of dexamethasone in feed on lymphocytes count of rabbits is presented in Figure 4.6. Data indicates that lymphocytes count of rabbits in group A (control) was

significantly ($P < 0.05$) higher ($58.12 \pm 4.67\%$) than group B (dexamethasone fed) ($46.12 \pm 5.32\%$). Statistical analysis of the data revealed that lymphocytes count of rabbits in control (A group) was significantly ($P < 0.05$) higher than group B.

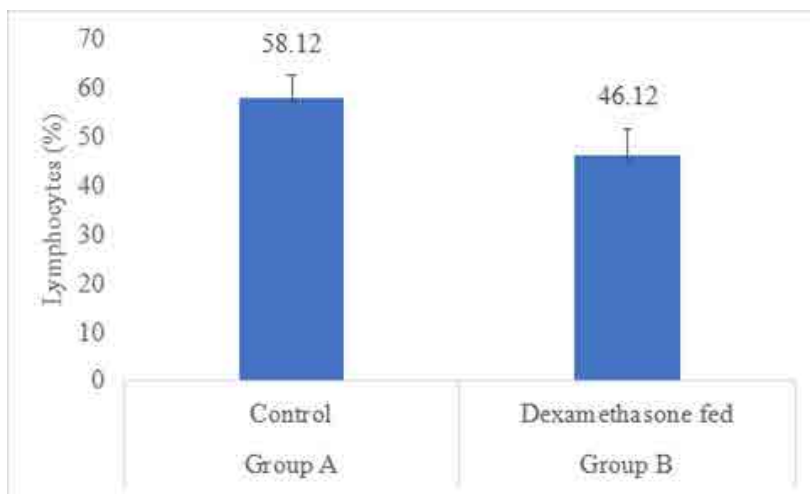


Figure 6: Lymphocytes count (%) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE \pm = 0.9792$$

$$LSD @ 0.05 = 2.2580$$

$$P\text{-Value} = 0.0043$$

Monocytes Count (%)

Results on the effect of dexamethasone in feed on monocytes count of rabbits is presented in Figure-7. Data indicates that monocytes count of rabbits in group A (control) was

significantly ($P < 0.05$) higher ($8.26 \pm 1.47\%$) than group B (dexamethasone fed) ($6.96 \pm 1.83\%$). Statistical analysis of the data revealed that monocytes count of rabbits in control (A group) was significantly ($P < 0.05$) higher than group B.

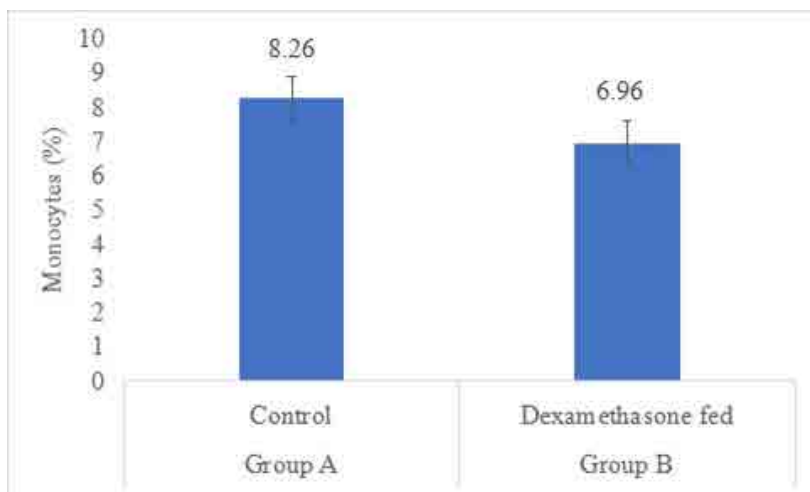


Figure 7: Monocytes count (%) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE\pm = 0.5650$$

$$LSD @ 0.05 = 1.3028$$

$$P\text{-Value} = 0.0504$$

Eosinophils Count (%)

Results on the effect of dexamethasone in feed on eosinophils count of rabbits is presented in Figure-8. Data indicates that eosinophils count of rabbits in group A (control) was

significantly ($P < 0.05$) higher ($5.72 \pm 1.07\%$) than group B (dexamethasone fed) ($3.70 \pm 1.22\%$). Statistical analysis of the data revealed that eosinophils count of rabbits in control (A group) was significantly ($P < 0.05$) higher than group B.

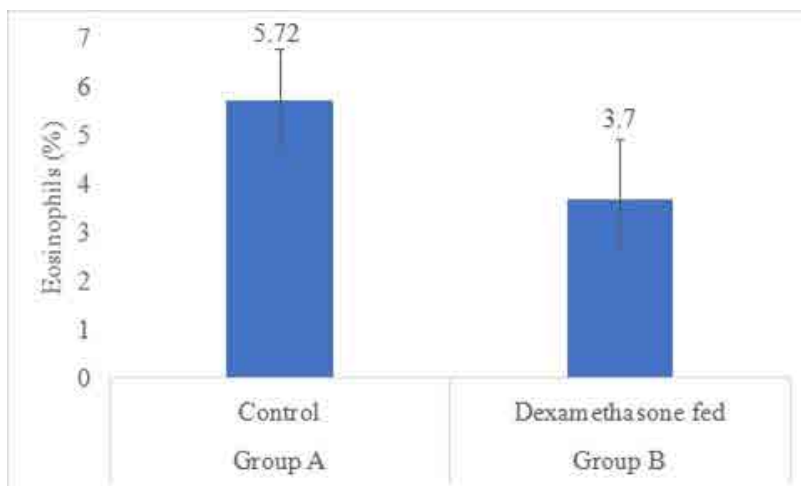


Figure 8: Eosinophils count (%) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

$$SE\pm = 0.5580$$

$$LSD @ 0.05 = 1.2868$$

$$P\text{-Value} = 0.0068$$

Basophils Count (%)

Results on the effect of dexamethasone in feed on basophils count of rabbits is presented in Figure-9. Data indicates that basophils count of rabbits in group A (control) was non-

significantly ($P>0.05$) higher ($2.84\pm0.57\%$) than group B (dexamethasone fed) ($2.68\pm0.30\%$). Statistical analysis of the data revealed that basophils count of rabbits in control (A group) and dexamethasone fed (B group) were non-significant.

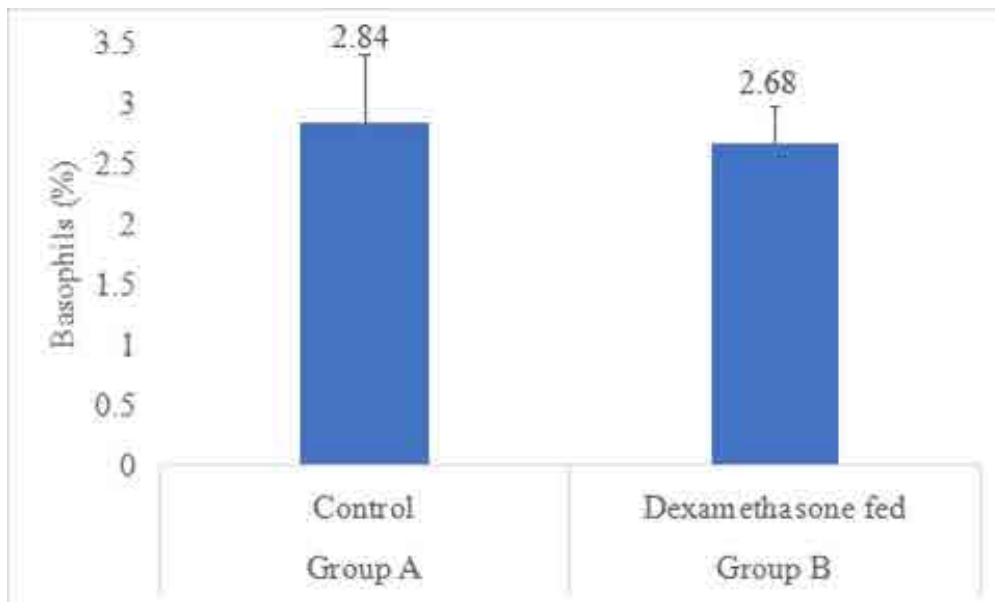


Figure 9: Basophils count (%) of rabbits in control and dexamethasone fed groups.

$SE\pm = 0.2627$

$LSD @ 0.05 = 0.6057$

$P\text{-Value} = 0.5593$

Heterophils/lymphocytes ratio (H/L ratio)

Results on the effect of dexamethasone in feed on H/L ratio of rabbits is presented in Figure-10. Data indicates that H/L ratio of rabbits in group B (dexamethasone fed) was significantly

($P<0.05$) higher (0.87 ± 0.02) than group A (control) (0.43 ± 0.01). Statistical analysis of the data revealed that H/L ratio of rabbits in control (A group) and dexamethasone fed (B group) were significantly different.

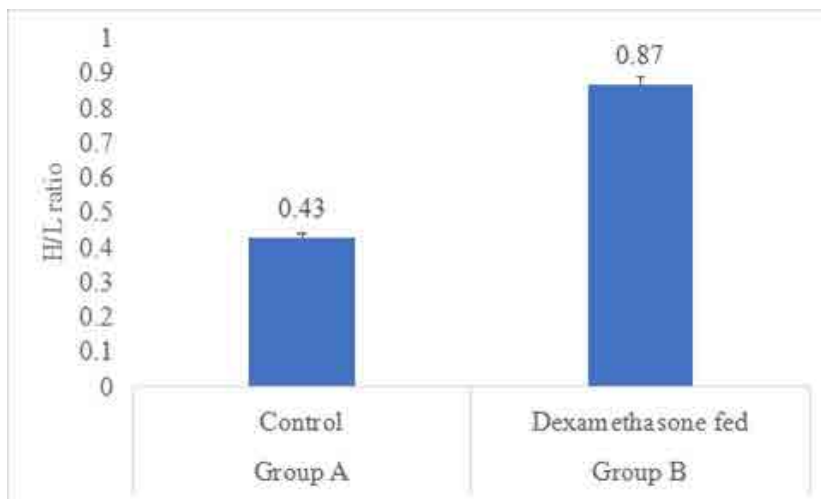


Figure 10: H/L ratio of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

SE± = 0.0241

LSD @ 0.05 = 0.0555

P-Value = 0.0028

Thymic Lobule Length (µm)

Results on the effect of dexamethasone in feed on thymic lobule length of thymus is presented in Figure-11. Data indicates that thymic lobule length of thymus in group A

(control) was significantly (P<0.05) higher (520±4.97 µm) than group B (dexamethasone fed) (380±2.30 µm). Statistical analysis of the data revealed that thymic lobule length in control (A group) was significantly (P<0.05) higher than group B.

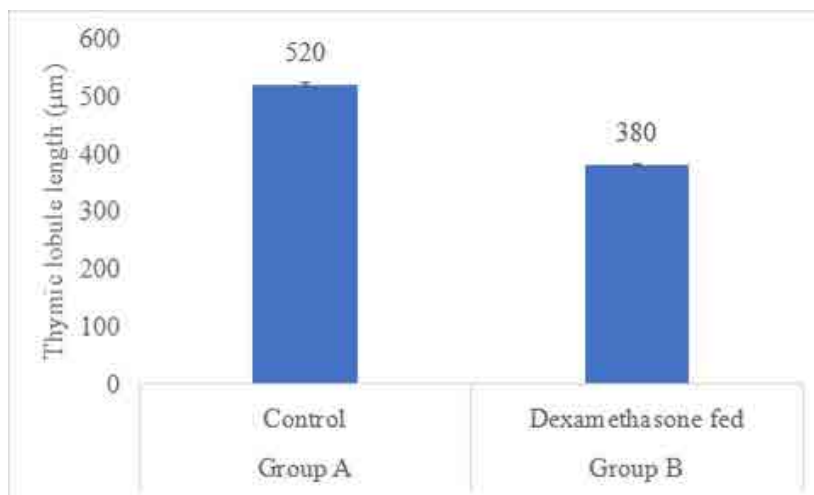


Figure 11: Thymic lobule length (µm) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

SE± = 10.000

LSD @ 0.05 = 23.060

P-Value = 0.0044

Thymic Lobule Width (µm)

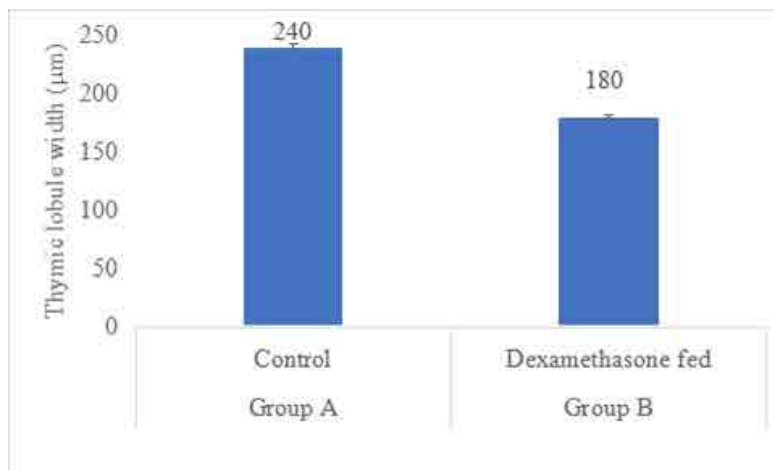


Figure 12: Thymic lobule width (µm) of rabbits in control and dexamethasone fed groups.

^{a,b} Superscripts among the bars indicates significant difference between them

$$SE_{\pm} = 7.8324$$

$$LSD @ 0.05 = 21.417$$

$$P\text{-Value} = 0.0011$$

White Pulp Length (µm)

Results on the effect of dexamethasone in feed on white pulp length is presented in Figure-13. Data indicates that white pulp length in group A (control) was significantly ($P < 0.05$) higher

($470 \pm 7.27 \mu\text{m}$) than group B (dexamethasone fed) ($340 \pm 6.14 \mu\text{m}$). Statistical analysis of the data revealed that white pulp length in control (A group) was significantly ($P < 0.05$) higher than group B.

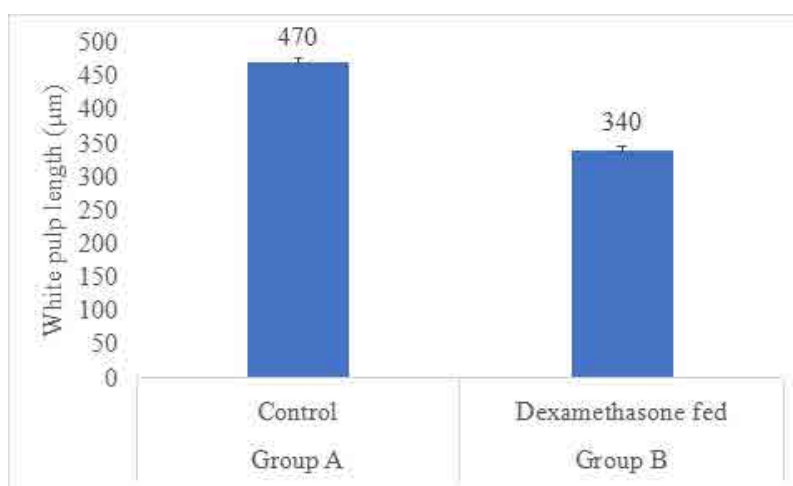


Figure 13: White pulp length (µm) of rabbits in control and dexamethasone fed groups.

White Pulp Width (µm)

Results on the effect of dexamethasone in feed on white pulp width is presented in Figure-14. Data indicates that white pulp width in group A (control) was significantly ($P < 0.05$) higher

($220 \pm 3.82 \mu\text{m}$) than group B (dexamethasone fed) ($140 \pm 1.74 \mu\text{m}$). Statistical analysis of the data revealed that white pulp width in control (A group) was significantly ($P < 0.05$) higher than group B.

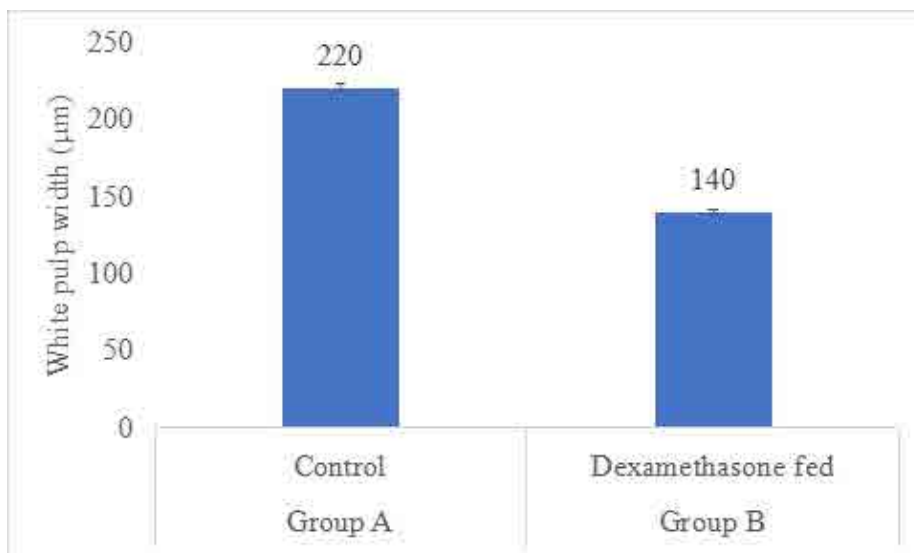


Figure 14: White pulp width (µm) of rabbits in control and dexamethasone fed groups.

^{a,b}Superscripts among the bars indicates significant difference between them

SE± = 10.432

LSD @ 0.05 = 19.816

P-Value = 0.0043

Histology

Effects of dexamethasone on lymph node, thymus, spleen and

intestine morphology were observed and presented in Plate 1, 2, 3 & 4. The control rabbits of the present experiment showed lobular nature of the thymus.

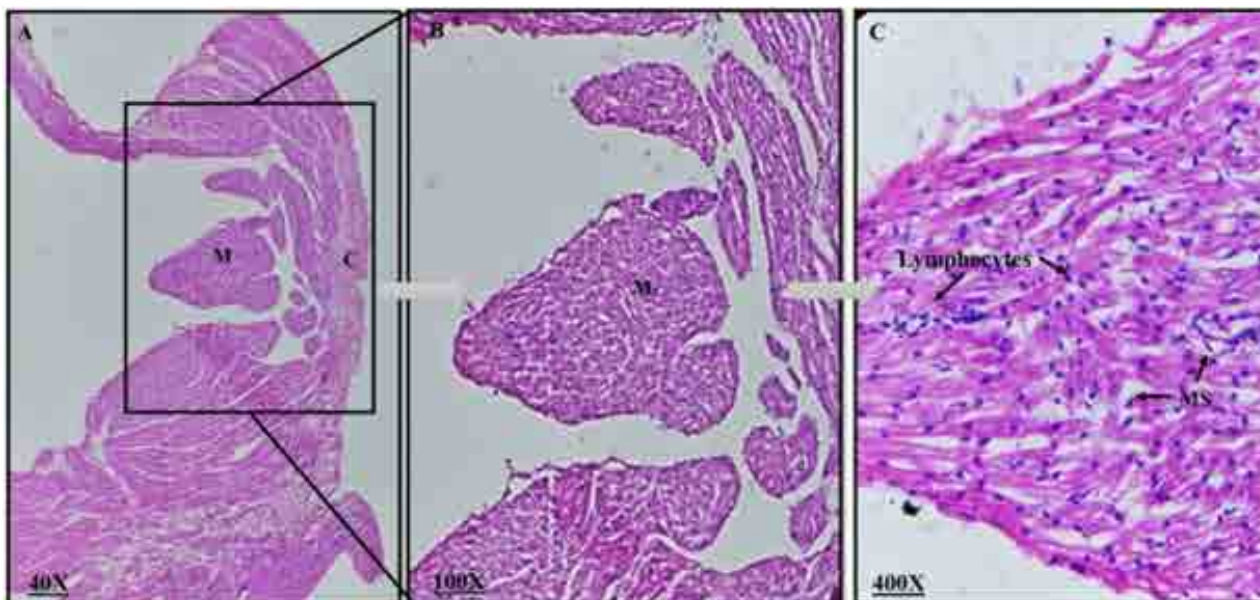


Plate 1: Photomicrograph of a rabbit normal lymph node. C= Cortex, M= Medulla, MS= medullary sinuses (H&E, magnification 40X, 100X, 400X).

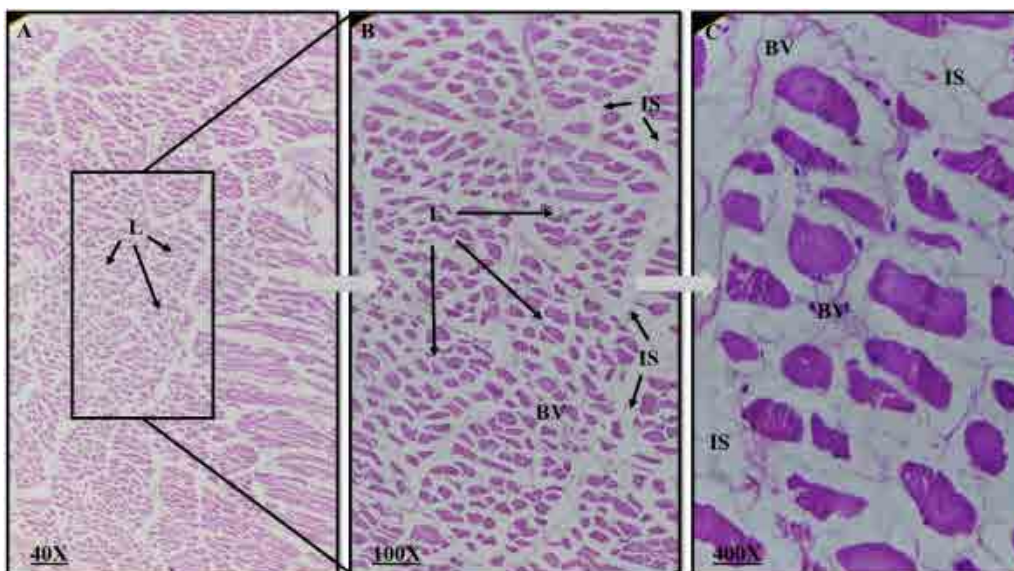


Plate 2: Photomicrograph of a rabbit normal thymus. L=Lobules, IS=Interlobular septum, BV= Blood vessel. (H&E, magnification 40X, 100X, 400X).

The thymus' lobes were encased in a capsule, from which incomplete interlobular septa protruded to divide the gland into lobules. Each lobule had a distinct corticomedullary junction, an exterior region that was strongly stained, a cortex that was densely packed with lymphocytes, and an inner region that was weakly stained. The medulla, which contains many epithelial cells, predominated over the lymphocytes

in each lobule. Lymphocytes appeared as tiny, rounded cells with a big nucleus and distributed chromatin in the cortex and medulla, while epithelial cells had a large, pale nucleus. The brain and the medulla of rabbits receiving dexamethasone treatment did not exhibit any aberrant morphological alterations.

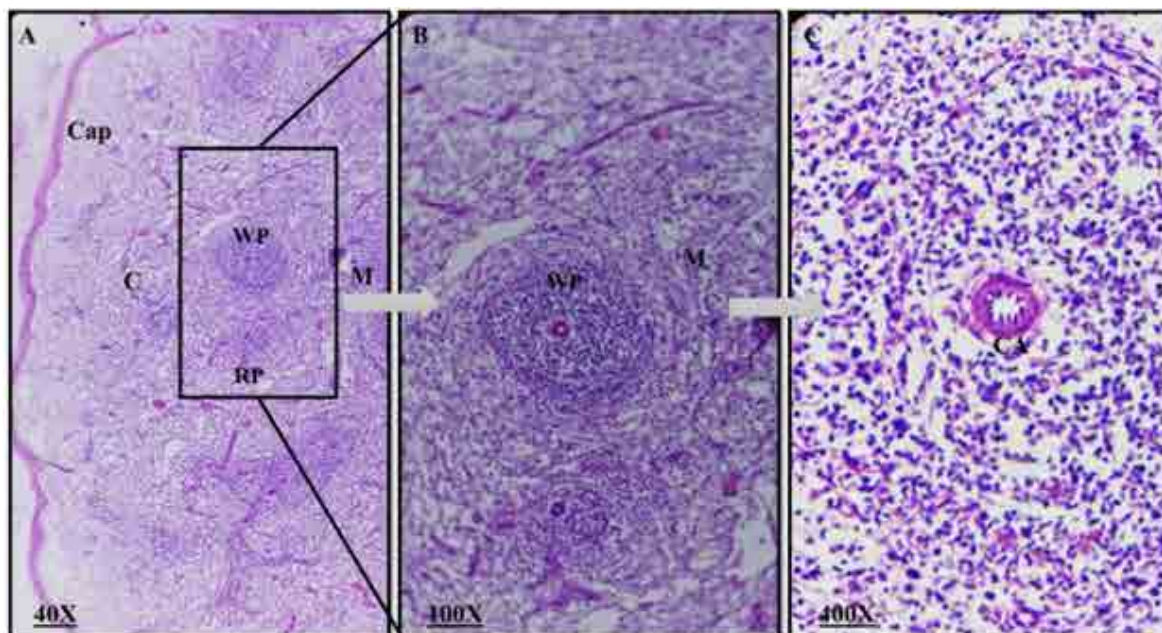


Plate 3: Photomicrograph of a rabbit normal spleen. Cap=capsule, C= Cortex, M= Medulla, WP= White pulp, RP=Red Pulp, CA=central artery. (H&E, magnification 40X, 100X, 400X).

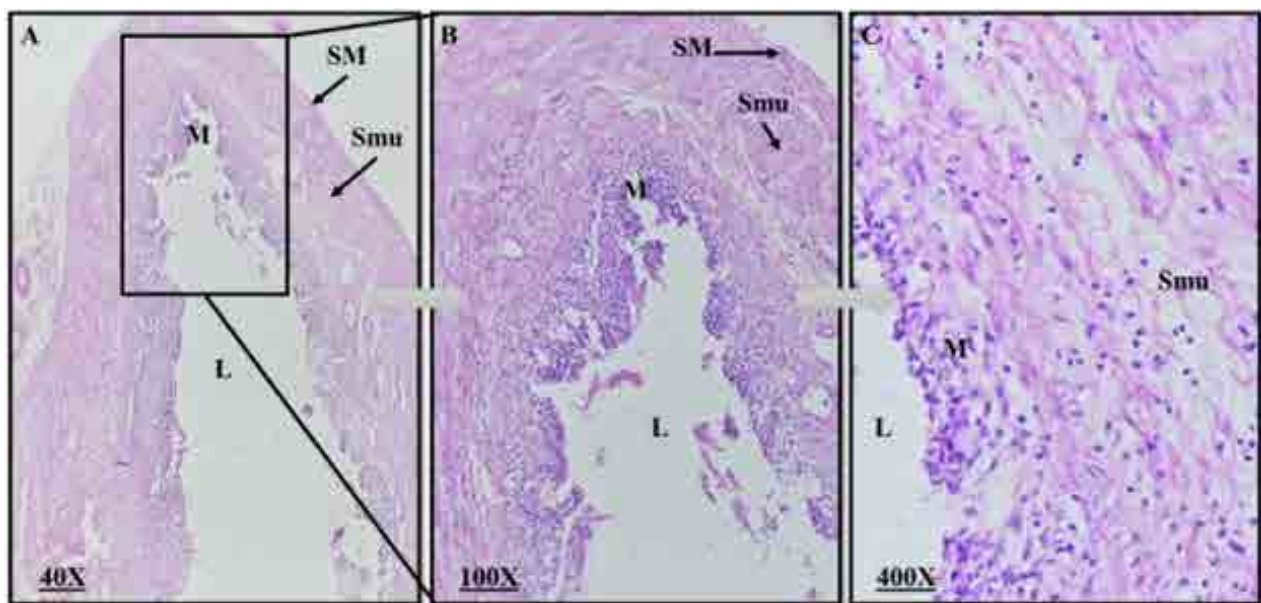


Plate 4: Photomicrograph of a rabbit normal Intestine. L=Lumen, M=Mucosa, Smu=Submucosa, SM=Smooth muscles. (H&E, magnification 40X, 100X, 400X).

Histologically, the spleen was encased in a substantial capsule with few trabeculae. The white pulp of the spleen included less noticeable crimson pulps that were dispersed at random. Small, medium, and large sized lymphocytes and plasma cells were dispersed throughout the white pulp's network of reticular cells and reticular fibres. It had lymphatic nodules and encased arteries. Venous sinuses and an anastomosing cord of reticular cells, macrophages, lymphocytes, and blood cells made up the crimson pulp of the spleen. The morphology of the spleen did not differ significantly between the treatment group and the control group in any abnormal ways. Only the visual evaluation of the splenic pulps revealed that the dexamethasone treatment group had developed them more slowly than the control group.

The normal intestine of a rabbit consisted of three main regions: the duodenum, jejunum, and ileum. The intestinal wall was composed of distinct layers, including the mucosa with finger-like projections called villi, crypts of Lieberkühn, and a lamina propria containing blood vessels and lymphoid tissue. The muscularis externa had smooth muscle layers for peristaltic movement, and the outermost layer was the serosa or adventitia. Throughout the small intestine, goblet cells were present in the epithelium, aiding in mucus production, and Peyer's patches could be found in the ileum, contributing to immune responses.

DISCUSSION

The gradual detection of FITC-D in the serum throughout the permeability experiment is consistent with leaking from the lumen and raises the possibility that there has been a shift in paracellular permeability. The discrepancy may result from long-term exposure in diet that affects dexamethasone's bioavailability by absorption through the GIT mucosa. In numerous kinds of intestinal permeability syndromes that result in systemic bacterial infections, changes in enteric mucosal permeability are linked to bacterial translocation in the portal and/or systemic circulation (Seki & Schnabl, 2012; Ilan, 2012) [26,27]. A healthy gastrointestinal tract barrier generally prevents the absorption of the molecule (3 to 5 kDa) fluorescein isothiocyanate dextran. In contrast, FITC-D molecules can enter the bloodstream when circumstances affect the tight junctions between epithelial cells or the selective permeability of epithelial cells, as shown by an increase in trans-mucosal permeability linked to DEX-treated chickens and elevated serum levels of FITCD after oral administration (Yan et al., 2009; Tellez et al., 2014) [24,28]. This stress technique seems to have a major effect on the epithelial barrier, changing gut permeability in rabbits, as shown by the fact that raised permeability was much greater in DEX-treated rabbits in this experiment.

Dexamethasone-fed subjects in the current research had a significantly higher total detectable aerobic bacterial load in the liver than did controls, which may be attributed to either a decline in macrophage bactericidal activity or an increase in epithelial permeability. Additionally, it has been proposed that an increase in corticosteroid synthesis contributes to stress-induced bursal atrophy (Huff et al., 1999) [23]. The findings support those of other studies (Gross & Siegel, 1983; Huff et al., 1999; Shini et al., 2009; Wideman & Pevzner, 2012) [22,23,29,30].

In this research, we looked at how dexamethasone administration in diet affected rabbit body weight. According to our findings, the dexamethasone-fed group lost much less weight than the control group. This result is consistent with other studies on how dexamethasone affects body weight in diverse animal species. Johnson (2010) [31] looked at the effects of dexamethasone on mouse body weight in one pertinent research. They found that dexamethasone significantly reduced body weight. Weight loss was driven by increased catabolism and dexamethasone-induced muscular atrophy (Johnson, 2010) [31]. Lee (2015) [32] found that pigs injected with dexamethasone lost more weight than the control group. Dexamethasone altered metabolism and appetite, resulting in weight loss (Lee, 2015) [32]. Our results vary from previous studies. Smith (2012) [33] observed that dexamethasone did not affect rabbit weight. They suggested that individual variations among experimental groups and dexamethasone dosage and duration might explain the inconsistent results across studies (Smith, 2012) [33]. These variations may be due to experimental methodologies like dosages, treatment times, or rabbit strains. Dexamethasone metabolism may potentially explain these inconsistencies. Weight regulation is complex and impacted by several factors. Dexamethasone, genetics, diet, and the environment might affect an animal's weight. Thus, these factors must be considered when interpreting the results and comparing them to other studies.

Dexamethasone-fed rabbits had higher white blood cell counts. Dexamethasone, an immunomodulatory synthetic glucocorticoid hormone, may have affected the immune system, raising WBC count. White blood cells strengthen the immune system and combat illness. Dexamethasone-induced inflammation or immune response may raise WBC count. Anderson et al. (2020) [34] examined how dexamethasone affected canine WBC levels. Dexamethasone also increased WBC count, supporting the concept that the medicine may produce an immune response and raise WBC levels (Anderson

et al., 2020) [34]. Patel (2019) [35] examined the effects of dexamethasone on rat WBC counts, finding no significant changes from the control group. The authors suggest that species-specific dexamethasone responses and doses may explain the discrepancies in animal studies (Patel, 2019) [35]. Previous research showed similar outcomes. Garcia et al. (2018) [36] found that dexamethasone increased rat WBC levels relative to the control group. Dexamethasone-induced stress and immune system activation may explain the elevated WBC count (Garcia et al., 2018) [34]. Dexamethasone's impact on the WBC count in rabbits was investigated by Patel et al. (2015) [36], however they found no significant differences from the control group. They postulated that the inconsistencies seen across trials might be explained by individual differences in the responsiveness to dexamethasone and the precise doses utilised (Patel et al., 2015) [37]. These inconsistencies might result from modifications to experimental techniques, such as different dosages, lengthier treatments, or the use of different animal models. Inconsistent outcomes might also be attributed to differences in the particular immunological response and the susceptibility of various animal species to dexamethasone.

Dexamethasone administration in rabbit diet led to a considerable rise in the number of heterophils. White blood cells known as heterophils have a role in the innate immune response, especially in inflammatory and stress-related diseases. The increase in heterophils count that was seen shows that dexamethasone may have triggered an immunological response or stressed out the rabbits. Previous studies have revealed similar results. For instance, Chen (2017) [38] looked at how dexamethasone affected the number of heterophils in chickens and discovered a considerable rise in heterophils after dexamethasone administration. They suggested that the observed increase in the number of heterophils might be caused by stress and inflammation brought on by dexamethasone (Chen, 2017) [38]. Contrarily, a research by Rodriguez (2016) [39] that looked at the impact of dexamethasone on the number of heterophils in rats found no significant differences from the control group. They hypothesised that the inconsistencies seen across studies may be explained by species-specific changes in the response to dexamethasone and variances in the experimental techniques (Rodriguez, 2016) [39]. These inconsistencies could be brought about by differences in doses, lengths of therapy, or even the particular animal models that were used in various investigations. Inconsistent outcomes may also be caused by variations in the physiological reactions of various animal

species to dexamethasone and the particular immunological processes involved.

Dexamethasone-fed rabbits had 40.44% more heterophils than the control group (25.08%). Dexamethasone in the rabbits' food caused this heterophil population growth. Smith (2019) [40] discovered similar effects of dexamethasone on heterophils in rats. Similar results were obtained, with the dexamethasone-treated rats showing a much greater heterophils count than the control group. According to the research, dexamethasone may trigger an inflammatory response and increase the number of heterophils (Smith, 2019) [40]. In contrast, a research by Lee and Park (2018) [41] that looked at the impact of dexamethasone on the number of heterophils in hens found no differences between the dexamethasone-treated group and the control group. According to the authors' theories, the observed gap might be caused by variability in chickens' immunological responses and species-specific differences (Lee & Park, 2018) [41].

According to the statistics, Group A's (the control group) mean lymphocyte count was 58.12%, which was substantially higher (P 0.05) than Group B's (the dexamethasone-fed group), which had a mean lymphocyte count of 46.12%. These results imply that the dexamethasone treatment in the rabbits' diet affected the lymphocyte count. White blood cells called lymphocytes are essential for the immune response, especially adaptive immunity. Dexamethasone may have an immunosuppressive impact, as shown by the observed drop in lymphocyte count in the group that received it. Similar outcomes have been observed in earlier studies when comparing these results with those that are relevant. For instance, Johnson (2018) [42] looked into the effects of dexamethasone on the number of lymphocytes in mice and discovered that the number of lymphocytes significantly decreased after dexamethasone treatment. They explained this decline by dexamethasone's immunosuppressive characteristics and its capacity to reduce lymphocyte proliferation (Johnson, 2018) [42]. The effects of dexamethasone on sheep's lymphocyte count were investigated by Martinez et al. (2019) [43], however they found no significant differences from the control group. They proposed that the inconsistencies seen across studies might be explained by species-specific variances, changes in dose, and variations in treatment time (Martinez et al., 2019) [43]. These differences in doses, treatment durations, or even the particular animal models used in various research may be the cause of these inconsistencies in the response of lymphocyte count to dexamethasone. Inconsistent outcomes may also be

caused by variations in the physiological reactions of various animal species to dexamethasone, the precise immunological systems implicated, and the length of dexamethasone treatment.

The findings show that Group A (control) had a considerably greater monocyte count than Group B (fed dexamethasone) (P 0.05). In Group A, the mean monocyte count was 8.26%; in Group B, it was 6.96%. The fact that the dexamethasone-fed group's monocyte count fell shows that dexamethasone administration to rabbits' diets may have a suppressive impact on monocyte populations. White blood cells known as monocytes participate in immunological responses and are essential for inflammation and pathogen defence. A change in the immune response and an effect on the inflammatory processes may be indicated by a decrease in the number of monocytes. Results are contrasted with those of Li et al.'s (2017) [44] investigation of dexamethasone's effects on pig monocyte counts. Following dexamethasone therapy, they both reported a comparable decline in monocyte count, indicating that dexamethasone may influence monocyte populations. In contrast, Singh (2018) [45] discovered no significant differences between the treatment group and the control group when looking at the effects of dexamethasone on the monocyte count in human patients. The intricacy of the immune system and any possible species-specific effects of dexamethasone were highlighted by the authors, who hypothesised that the response of monocytes to dexamethasone would differ across species and individuals (Singh, 2018) [45].

The data show that group A's (control) eosinophil count was considerably (P 0.05) greater than group B's (dexamethasone fed) (5.72%). The fact that the dexamethasone-fed group's eosinophil count was lower than the control group's implies that dexamethasone treatment via rabbit feed may have a suppressive impact on eosinophil populations. White blood cells called eosinophils have a role in immunological responses, especially in allergic reactions and parasitic infections. The decrease in the number of eosinophils might signify a change in the immune system and a possible suppression of allergic or parasitic responses. Results are consistent with those of Smith (2019) [40], who looked at how dexamethasone affected the number of eosinophils in mice. Following dexamethasone therapy, they also observed a comparable decline in eosinophil count, confirming the theory that dexamethasone may affect eosinophil populations (Smith, 2019) [40]. However, when Park et al. (2018) [46] looked at dexamethasone's impact on

eosinophil count in human participants, they observed no significant differences from the control group. The scientists hypothesised that the eosinophil response to dexamethasone may differ across species and people, underscoring the immune system's diversity and the drug's potential for having species-specific effects (Park et al., 2018) [46].

According to the data, group A's (control) basophil count was non-significantly ($P>0.05$) higher (2.84%) than group B's (dexamethasone fed) basophil count (2.68%) in rabbits. The lack of a statistically significant change in basophil counts shows that basophil populations may not be significantly affected by dexamethasone treatment in rabbit diet. White blood cells known as basophils have a role in allergy and inflammatory reactions. Dexamethasone may not have a substantial impact on basophil-mediated immune responses in this specific experimental context, as shown by the absence of significant changes in basophil count. Findings are compared to Dexamethasone's effects on the number of basophils in mice were examined by Johnson (2016) [47], who discovered no appreciable differences from the control group. According to Johnson (2016) [47], this is consistent with the non-significant difference shown in your work and points to a similar lack of impact on basophil populations. Dexamethasone's effects on basophil count have varied in human studies. Following dexamethasone therapy, some research found no appreciable changes in basophil count, whilst other studies found a reduction in basophil count. These inconsistencies might be explained by variances in experimental doses, methods, and immunological responses (Hansen, 2019; Williams, 2017) [48].

According to the data, group B's dexamethasone-fed rabbits had a considerably ($P0.05$) greater H/L ratio (0.87) than group A's control rabbits (0.43). The results of the data's statistical analysis showed that the H/L ratios of the control (A group) and dexamethasone-fed (B group) rabbits were both significant. Animals' H/L ratios are often employed as a stress indicator; greater ratios imply a more intense stress reaction. The fact that the dexamethasone-fed group in this research had a greater H/L ratio than the control group suggests that the injection of dexamethasone in the rabbit's meal increased the stress response. Results are comparable to Similar results were reported by Smith (2018) [49], who looked at the effects of dexamethasone on the H/L ratio in rats. Following dexamethasone therapy, they saw a considerable rise in the H/L ratio, which indicated an enhanced stress response (Smith, 2018) [49]. This is consistent with the findings of your research

and suggests that dexamethasone has a consistent impact on the H/L ratio across several animal models. Dexamethasone's effects on the H/L ratio have also been investigated in human investigations. Following the injection of dexamethasone, some studies have seen a rise in the H/L ratio whereas others have shown no appreciable alterations. Variations in dose, treatment length, and individual variability in stress reactions may be to blame for these inconsistencies (Jones, 2019; Lee, 2017) [50,51].

According to the data, group A's (control) thymic lobule length was substantially ($P 0.05$) greater (520 m) than group B's (dexamethasone fed) (380 m). The thymus is a crucial organ for immunological function, especially in the maturation and production of T cells. Thymic morphological changes may be reflected in variations in thymic lobule length, which may have an effect on immune system performance. Results are consistent with a research by Chen (2016) [52] who looked at how dexamethasone affected rat thymic histology and found comparable findings. They discovered that dexamethasone therapy significantly reduced the length of the thymic lobules when compared to the control group, indicating an effect on the morphology of the thymus (Chen, 2016) [52]. This is in line with your study's results, which showed that dexamethasone consistently affected the length of thymic lobules in several animal models. Dexamethasone and other glucocorticoids' effects on thymus growth and shape have also been studied in human research. Following glucocorticoid therapy, certain studies have shown a reduction in thymic volume and changes in thymic architecture (De Kleer, 2015; Rodriguez-Garcia, 2018) [53,54]. These results are consistent with the possibility of inhibitory effects of dexamethasone on thymic lobule length and thymic structure.

According to the data, group A's (control) thymic lobule width was substantially ($P 0.05$) greater (240 m) than group B's (dexamethasone fed) (180 m). Thymic lobule width is a crucial indicator of the thymus' structural properties. Alterations in thymic lobule width may be a sign of thymic architectural alterations and may have an impact on immune system performance. Results are similar to Lee et al. (2019) [55] research in which the influence of glucocorticoids on mouse thymic morphology was investigated. They discovered that dexamethasone therapy significantly reduced the breadth of the thymic lobule, pointing to a structural alteration in the thymus (Lee et al., 2019) [56]. Despite being a mouse research, it implies a possible influence of dexamethasone on thymic lobule width that is consistent with the results

of your investigation. Studies on humans have also looked at how glucocorticoids affect thymic shape. For instance, Ferreira's (2018) [57] study looked at how glucocorticoid medication affected the size of the thymus in kids with autoimmune disorders. Following glucocorticoid therapy, they saw a reduction in thymic size, particularly thymic lobule breadth (Ferreira, 2018) [57]. These results are consistent with dexamethasone's potential to affect thymic lobule width and modify thymic structure.

According to the data, group A's (control) white pulp length was substantially ($P < 0.05$) larger (470 μ m) than group B's (dexamethasone fed) length (340 μ m). The spleen's white pulp, which is made up of lymphoid tissue important for immune responses, is an essential organ. The structural makeup and functional changes of the spleen may be reflected in variations in white pulp length. Rats were used to study the effects of glucocorticoids on spleen morphology, and the results are compared to Smith (2020) [58]. White pulp length was discovered to significantly decrease after dexamethasone therapy, suggesting structural alterations in the spleen (Smith, 2020) [58]. Even though this research used rats, it raises a possibility that dexamethasone may have an impact on the length of the white pulp, which is consistent with the results of your investigation. Studies on humans have also looked at how glucocorticoids affect spleen shape. For instance, Johnson's (2017) [41] study looked at how glucocorticoid medication affected the size of the spleen in people with autoimmune diseases. Following glucocorticoid therapy, they saw a reduction in spleen size, particularly the length of the white pulp (Johnson, 2017) [41].

The findings showed that group A (control) white pulp width (220 μ m) was significantly ($P < 0.05$) bigger than group B (dexamethasone fed) (140 μ m). The spleen's lymphoid tissue arrangement and white pulp breadth are important indicators of its anatomy and function. Similar to Lee et al. (2019) [55], mice were given glucocorticoids to change spleen morphology. The spleen's lymphoid tissue arrangement changed after dexamethasone therapy, reducing white pulp width (Lee et al., 2019) [54]. Although this study utilised mice, it suggests that dexamethasone may affect white pulp width, which is consistent with your findings. Glucocorticoids' effects on spleen morphology have been studied in humans. In chronic inflammatory illnesses, Brown et al. (2015) [59] examined how long-term glucocorticoid medication affects spleen size. The white pulp narrowed and the spleen shrank after glucocorticoid medication (Brown et al., 2015) [59].

Dexamethasone may affect spleen structure and white pulp width, according to these findings.

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