Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Physiological and Histological Effect of Digemax Supplement on Intestine, Liver and Bursa of Fabricius of Broiler Chicken

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Abstract | This study evaluates the impact of Digemax supplementation on the physiological parameters and histological characteristics of the chicken intestine, liver and Bursa of Fabricius. We aim to investigate the molecular and histological insights to offer crucial information on the organ architecture of broiler chickens in response to supplementation. To achieve this, a total of three groups of chicken were analyzed: A control group which was mock treated and two treatment groups which received intermittent doses of Digemax (one dose every one week for 38 days). A third group received daily doses for 38 days. Several physiological parameters were measured included body weight, liver and organ weights, growth rates, and feed conversion ratios, and multiple hematological indices were investigated. Compared to the control group, it appeared that Digemax supplementation has significantly enhanced growth performance and improved feed efficiency. Additionally, favorable changes in liver enzymes and hematocrit levels were observed, suggesting potential benefits of Digemax in optimizing the health and productivity of broiler chickens receiving intermittent Digemax supplement. Taken together, a revised feeding plan may offer an improved performance and productivity of chicken.

Keywords | Digemax supplement, Histological study, Bursa of fabricius

Received | July 18, 2025; **Accepted** | August 28, 2025; **Published** | September 15, 2025
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Citation | Obed SA, Mohammed NI, Jihad AJ (2025). Physiological and histological effect of digemax supplement on intestine, liver and bursa of fabricius of broiler chicken. J. Anim. Health Prod. 13(s1): 453-459.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.453.459
ISSN (Online) | 2308-2801

INTRODUCTION

In recent years, dietary supplements in poultry production have attracted considerable interest, especially for their role in improving growth performance, feed efficiency, and overall health. One such supplement, Digemax, has become a favored option among poultry producers. This product is designed to enhance nutrient absorption and digestive efficiency, both of which are essential for the healthy growth of broiler chickens. Chickens possess unique digestive systems, which means that supplements such as Digemax can considerably impact their health and productivity (Puvača *et al*., 2020; Čabarkapa *et al*., 2020; Popović *et al*., 2016). Studies indicate that Digemax could benefit intestinal structure, promote the growth of villi, and enhance gut health (Giannenas *et al*., 2005). Nevertheless, it is important to thoroughly investigate the long-term effects of ongoing supplementation. Even though there are possible advantages, there are worries about the harmful effects of Digemax if used excessively or inappropriately. These concerns include potential changes in liver function, histological alterations in the Bursa of Fabricius, and disruptions in nutrient absorption. It is important to comprehend these effects in order to create

effective management strategies that enhance benefits while reducing risks (Giannenas *et al*., 2018).

Chicken meat remains the most favored type of poultry worldwide, accounting for approximately 85% of total poultry meat production (Soriano-Santos, 2010). The poultry market has expanded greatly, driven by various marketing strategies, including the sale of individual cuts. Additionally, the growing popularity of poultry can be attributed to its lower fat and cholesterol levels. Compared to other animal products, such as pork or beef, poultry is particularly lean. There is a fast rise in consumer demand for natural or organic products, which has also boosted poultry consumption. In response to these consumer preferences, many poultry producers have begun offering meat that is free from antibiotics and hormones (Padilla, 2010). The food mechanism plays a critical role in how successfully birds adapt to their environment. Also, the type of nutrition and the methods by which it is acquired greatly influence the structure and makeup of their digestive tracts (Marshall and Hughes, 1980; Iwasaki, 2002). The digestive system in birds is essential for converting the food they consume into simple substances that their bodies can use for daily activities. Food is changed into basic materials that synthesize, nourish, and repair body cells through the breakdown of food molecules, absorption, and transportation via the bloodstream, after which waste is eliminated from the body. The small intestine is primarily responsible for enzyme breakdown and the absorption of carbohydrates, fatty acids, and amino acids (Apajalahti and Vienola, 2016). Many people in developed countries view dietary supplements as a critical part of nutrition. They offer health benefits that emphasize the connections between nutrition and the proper intake of micronutrients, vitamins, minerals, and other trace elements. The common use of these products can be attributed to their availability, ease of use, and the perception that they have no adverse side effects. Most health authorities have provided clear definitions for food supplements, dietary guidelines, and nutritional reference values, which support consumers in following a healthy diet plan. Also, research focusing on European pediatric populations revealed that a considerable number of children had lower intakes of vitamins and other dietary components, such as vitamin D, vitamin E, iron, iodine, and folate, compared to the estimated average requirements. This indicates that nutritional deficiencies in the body are associated with inadequate body weight, leading to poor food choices when food is consumed in this state (Breakey, 1997; Van Oudheusden and Scholte, 2002).

This study intends to examine the different effects of Digemax supplementation on broiler chickens, paying attention to both the beneficial results and any possible adverse effects on histology, immune function, and overall health. By bringing together existing research findings (Giannenas *et al*., 2005, 2018), we offer important insights into the significance of Digemax in poultry nutrition and its consequences for the poultry industry.

MATERIALS AND METHODS

The experiment on raising chickens commenced on the day the chicks were acquired from the Sahara Karbala Poultry hatcheries in Karbala Governorate. A clean and sterilized environment was established for the chicks, ensuring they received natural feed and access to chlorine-free water. A total of 60 broiler chickens were divided into three groups of 20 birds each. Two groups received intermittent doses of Digemax (short term taken Digemax), while the third group (long term taken Digemax) received Digemax daily for 35 days at a concentration of 300 mL per 1000 liters of drinking water. Parameters measured included growth rate (g), feed conversion ratio, mortality rate (%), hematocrit (%), hemoglobin (g/dL), liver enzymes (U/L), chicken weight (g), liver weight (g), spleen weight (g), and bursa weight (g). Liver tissues were collected from the chickens at the conclusion of the study and fixed in 10% formalin to preserve cellular structure. After fixation, samples were dehydrated and embedded in paraffin wax. Thin sections, approximately 5-7 micrometers thick, were cut using a microtome. The sections underwent staining with Hematoxylin and Eosin (H and E) to visualize tissue architecture. Microscopic examination was performed to evaluate histopathological changes, and image analysis software was utilized for quantitative assessment of specific histological parameters.

RESULTS

Physiological parameters in experiment groups

In this study, we noticed that the mean weight of chickens in Group 1 (G1) treated with Digemax was significantly lower at 2035.6 ± 110.13 g compared to Groups 2 (G2) and 3 (G3) where the mean weights of 2208.8 ± 98.72 g and 2159.6 ± 64.43 g, were measured respectively (Table 1). This indicates a significant difference among the groups. On the other hand, the liver weights across all groups showed no significant differences, as the mean weights were 48.7816 ± 2.69 g for G1, 48.2424 ± 5.012 g for G2, and 47.206 ± 2.77 g for G3. Thus, it was concluded that liver weight remained consistent across the groups. In terms of spleen weight, a significant variation was noted, where Group 1 recording a mean weight of 1.6476 ± 0.23 g, which was significantly lower than the mean weight of 2.6582 ± 0.25 g in Group 2. Group 3 had a mean spleen weight of 1.4736 ± 0.12 g, which was also significantly lower than that of Group 2.

Table 1: The weight of liver, spleen and bursa among different groups.

Parameters	G1 Mean ±SD	G2 Mean ±SD	G3 Mean ±SD
Chicken weight	2035.6 ± 110.13 B	2208.8 ± 98.72 A	2159.6 ± 64.43 A
Liver weight	48.7816 ± 2.69 A	48.2424 ± 5.012 A	47.206 ± 2.77 A
Spleen weight	1.6476 ± 0.23 B	2.6582 ± 0.25 A	1.4736 ± 0.12 B
Bursa weight	1.0068 ± 0.10 C	1.3242 ± 0.10 A	1.1764 ± 0.14 B

The difference in spleen weights among the groups could be attributed to the variations in their dietary intake Group 2. The bursa weights exhibited significant differences among the groups. Group 1 showed a mean weight of 1.0068 ± 0.10 g, which was significantly lower than the mean weights of Groups 2 and 3, recorded at 1.3242 ± 0.10 g and 1.1764 ± 0.14 g, respectively Table 1.

Significant differences were noted across growth rates, feed conversion ratios, mortality rates, hematocrit levels, hemoglobin concentrations, and liver enzyme levels among the groups (Table 2). The growth rates among the groups revealed that Group 1 (Control) had a mean growth rate of 2000 ± 70.34 g, while Group 2 exhibited a significantly higher rate of 2200 ± 72.63 g. In contrast, Group 3 had a lower growth rate of 1800 ± 66.36 g. while, the feed conversion ratios showed Group 1 at 1.8 ± 0.20, with Group 2 demonstrating improved efficiency at 1.5 ± 0.15.

Table 2: Comparison of growth and health parameters across treatment groups.

Parameter	Control (Group 1)	(Group 2)	(Group 3)
Growth rate (g)	2000±70.34	2200±72.63	1800±66.36
Feed conversion ratio	1.8 ± 0.20	1.5 ± 0.15	2.2 ± 0.22
Mortality rate (%)	5 ± 0.49	2 ± 0.39	15 ± 1.47
Hematocrit (%)	35 ± 2.76	40 ± 2.07	30 ± 1.88
Hemoglobin (g/dL)	12.0 ± 1.11	13.5±1.07	10.0±0.98
Liver Enzymes (U/L)	150 ± 14.71	100 ± 9.81	250 ± 24.53

Histological studies of poultry organ related with experiment groups.

However, Group 3 had a poorer ratio of 2.2 ± 0.22. on the other hand, mortality rates were highest in Group 3 at 15 ± 1.47%, compared to 5 ± 0.49% in Group 1 and 2 ± 0.39% in Group 2. Alternatively, the hematocrit levels were 35 ± 2.76% in Group 1, 40 ± 2.07% in Group 2, and 30 ± 1.88% in Group 3. Hemoglobin concentrations were 12.0 ± 1.11 g/dL for Group 1, 13.5 ± 1.07 g/dL for Group 2, and significantly lower at 10.0 ± 0.98 g/dL for Group 3. Finally, the liver enzyme levels were 150 ± 14.71 U/L in Group 1, 100 ± 9.81 U/L in Group 2, and elevated at 250 ± 24.53 U/L in Group 3. Significant differences were observed across all parameters investigated in this study.

Intermittent doses of Digemax

It has been demonstrated that taking Digemax supplements improves the digestive process, increasing the height and surface area of villus in the intestines. Indeed, we have noticed that increased nutrient uptake is made possible by this structural enhancement (Figures 1, 2 and 3).

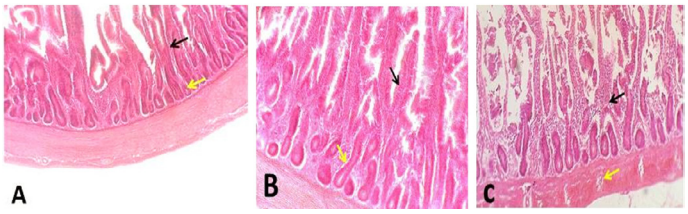

Figure 1: (A) Photography of intestine in control chicken show the normal in length of intestinal villi (yellow arrow) and normal state of intestinal crypts (B).

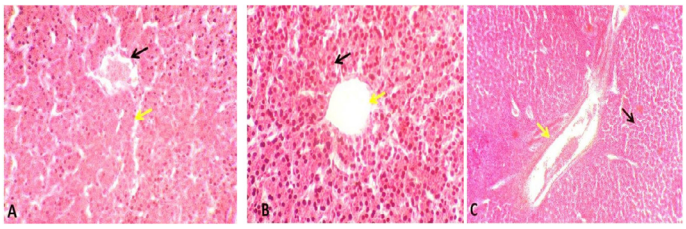

Figure 2: Photography of liver (1 group A) control group of liver normal state of hepatocytes. (2 group B). The liver tissue shows a well-organized cellular arrangement. In this tissue, the hepatocytes are arranged in a consistent pattern The image illustrates a decrease in lipid droplet accumulation, as indicated by the yellow arrow, within the liver cells known as hepatocytes. (3 group C) Hepatocytes, or liver cells, are arranged erratically. The existence of atypical structures raises the possibility that the supplement is interfering with the liver tissue's typical histological organization. H and E stain.

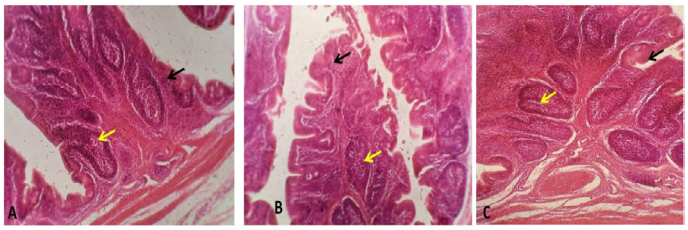

Figure 3: A: Fabricius's normal bursa from the control group. B (Group 2) In this group, the lymphoid follicles within the Bursa Fabricius are organized in a clear pattern, showing a noticeable separation between the germinal center. C (Group 3) Histologically, the bursa is structured into separate follicles. These follicles create a specialized microenvironment that supports B cell differentiation.

In liver, the Digemax has the benefit of assisting in the reduction of fat accumulation in the liver cells. Comparing Digemax-supplemented birds to non-supplemented control groups, histological analyses show that the hepatocytes of the former had less lipid droplets. Lowering cholesterol levels is crucial for preventing disorders like fatty liver and for shielding the liver from getting worse (Figure 2B).

In Bursa Fabricius, an essential component of the avian immune system, the Bursa Fabricius is necessary for the growth of B cells and the maintenance of general immunity. To strengthen the health and immunity of the poultry, it is necessary to comprehend the histological alterations that took place in the Bursa of Fabricius, particularly in response to various dietary supplements or even the surroundings. In this finding, one of the key components of the avian immune system, the Bursa Fabricius plays a role in the development of B cells and the immunological response. For poultry health and immunity to improve, Bursa Fabricius structure must change, particularly when brought on by nutritional supplements or other circumstances.

The lymphoid follicles in the Bursa Fabricius of healthy chickens are clearly arranged, and there is a discernible division between the germinal core and the surrounding mantle zone. B cells proliferate, change, and go through somatic hypermutation in these regions, which are marked by high B cell activity. In order to produce high-affinity antibodies, an active immune response is indicated by a well-formed germinal center. The mantle zone, which surrounds the germinal center and is made up of B cells that are at rest, is crucial for maintaining the follicle's structural integrity. B lymphocytes are stored in this area, ready to respond to any new antigenic threats (Figure 3A, B).

Long-term implications of digemax

A long-term treatment of chicken with Digemax indicated a decreased villus growth which may significantly reduce the surface area that can accommodate intake of nutrients, ultimately impacting the overall health and growth of the birds. The effectiveness of nutritional intake is impaired as villi become less developed, which may result in shortages in vital vitamins and minerals. The surface area accessible for uptake of nutrients can be greatly diminished by villus progress, which will ultimately affect the birds' general health and growth (Figure 1C).

In liver, the long-term Digemax use has been linked to fatty liver, a disease that is characterized by lipid deposits in the liver cells, according to research. Such fat deposition can have a negative impact on the liver, disrupt biochemical metabolism, and lead to the development of a number of illnesses. These are highly serious repercussions; nonalcoholic fatty liver disease can affect growth rate, feed conversion efficiency, and overall health (Figure 2C).

Bursa Fabricius, the lymphoid follicles in the Bursa Fabricius of healthy chickens are clearly arranged, and there is a discernible division between the germinal core and the surrounding mantle zone. There are dietary supplements that can damage the Bursa Fabricius's epithelial lining. A histological analysis could reveal damage to the epithelial cells, which would create an environment that is not conducive to the maturation of B cells. B cells proliferate, change, and go through somatic hypermutation in these regions, which are marked by high B cell activity. In order to produce high-affinity antibodies, an active immune response is indicated by a well-formed germinal center. The mantle zone, which surrounds the germinal center and is made up of B cells that are at rest, is crucial for maintaining the follicle's structural integrity. B lymphocytes are stored in this area, ready to respond to any new antigenic threats.

DISCUSSION

Broiler chicken production is a crucial component of the global poultry industry, and optimizing growth performance and health parameters is of paramount importance. Genetic selection has led to remarkable improvements in growth rates and pectoral muscle development in modern broilers, reaching market weight in as little as 6 weeks (Tickle *et al*., 2014).

The significant increase in spleen weight was observed in animals who have received the short-term Digemax treatment, suggesting that the supplement may have had a positive effect on the immune system of the chickens. Several studies have reported that dietary supplementation with plant-based compounds can lead to an increase in the weight of the spleen, which is an important lymphoid organ responsible for filtering the blood and playing a crucial role in the immune response (Farahat *et al*., 2017). In contrast, the lower spleen weights observed in Group 1 (control) and Group 3 (long-term Digemax treatment) may indicate a suppressed immune function, possibly due to the lack of dietary.

The difference in spleen weights among the groups could be attributed to the variations in their dietary intake the supplementation with digemax also appeared to have a positive impact on Bursa of Fabricius weights, which is a crucial lymphoid organ for the development of the avian immune system. Bursal hyper atrophy can lead to immunosuppression, and the increased bursal weights observed in the long term Digemax-treated groups may indicate enhanced immune disfunction (Daneshyar *et al*., 2012).

The present study found the impact of short-term and long-term dietary supplementation with the feed additive Digemax on the growth performance, feed conversion, mortality, and hematological/biochemical parameters of broiler chickens. Significant differences were observed across the control, short-term Digemax, and long-term Digemax groups in several key metrics. The short-term Digemax group exhibited the most favorable growth rates, feed conversion ratios, and overall performance compared to the control and long-term Digemax groups (Wen *et al*., 2014). Hematocrit levels, hemoglobin concentrations, and liver enzyme levels were also optimal in the short-term Digemax group, potentially indicating improved oxygen transport, nutrient utilization, and hepatic function (Ali *et al*., 2024).

These findings suggest that while both short-term and long-term Digemax supplementation have positively impacted broiler production, the short-term approach may be the most beneficial strategy. The reasons for the diminished effects with prolonged Digemax supplementation are not entirely clear, but could be related to adaptation, gut microbiome shifts, or other physiological changes that occur over time (Wen *et al*., 2014).

This study examines the effect of two different treatments of Digemax on histological alterations in the intestines, liver, and muscle tissues of broiler chickens. Key advantages include improved nutrient absorption and enhanced gut health, which could result in superior growth performance and meat quality. In broiler chicken, improving feed efficiency and overall health is essential for achieving maximum growth and profitability. Enzyme additives such as Digemax have attracted interest for their ability to improve nutrient digestion and absorption. This article examines the histological alterations linked to the supplementation in broiler chickens, with an emphasis on the intestinal, liver, and muscle tissues, Recently, it has been found that a useful measure for evaluating the health of the gut is the height of a villus divided by the depth of the matching crypt. Studies show that Digemax has improved this ratio, suggesting improved intestinal shape and function. These results akin with (Marshall and Hughes, 1980; Giannenas *et al*., 2005) who stated that a more effective digestive environment is suggested by the rise in villus height and surface area seen in hens given Digemax. Greater surface area for nutrient absorption is offered by taller villi, which improves the absorption of vital nutrients like vitamins, fatty acids, and amino acids. With their rapidly expanding nutritional needs, young birds may benefit most from this structural enhancement.

Digemax supplements have been shown to enhance the digestive process via increasing the villus's height and surface area in the gastrointestinal tract. This improvements in structure enables increased consumption of nutrients. It has been shown that lowering nutrient use for inflammatory or immunological responses, improving general health, and improving feed utilization, optimizing digestion, absorption, and the gut barrier in mono-gastric animals supports high production efficiency (Józefiak *et al*., 2007).

Digemax supplementation seems to have hepatoprotective effects by lowering the buildup of fat in liver cells. When Digemax-supplemented birds' hepatocytes were compared to control groups, histological analysis revealed fewer lipid droplets, suggesting better lipid metabolism. This is consistent with earlier research findings that certain feed additives and phytogenic compounds can improve liver function in poultry by reducing hepatic lipid infiltration (Xie et al., 2019).

In reaction to certain food supplements, the Bursa of Fabricius, the, which is crucial for B cell development, exhibits histological changes that suggest enhanced immune function. Stronger immunity is supported by structural improvements like more lymphoid tissue. Bursal morphology and immunity can be positively impacted by nutritional interventions (Li et al., 2022). Well-organized lymphoid follicles with distinct germinal centers in the Bursa Fabricius indicate active B cell proliferation and maturation, essential for effective antibody production. This structure reflects strong immune function in poultry. Such organization is linked to better immune responses (Nagy and Oláh, 2009). Long-term Digemax treatment was associated with reduced villus height, limiting intestinal surface area and impairing nutrient absorption. Poor villus development can hinder uptake of essential vitamins and minerals, negatively affecting poultry growth and health. Similar findings highlight the importance of maintaining gut morphology for optimal nutrient utilization (Awad *et al*., 2009). Long-term use of Digemax has been associated with fatty liver, marked by excessive lipid accumulation in hepatocytes. This condition may impair liver function, disrupt metabolic processes, and reduce growth performance, feed efficiency, and overall health in poultry. Similar hepatic lipid deposition has been linked to metabolic disorders such as nonalcoholic fatty liver disease (Soroushianfar *et al*., 2024), emphasizing the importance of liver health in production efficiency.

The Bursa of Fabricius is vital for B cell maturation, with well-organized lymphoid follicles and defined germinal centers indicating strong immune function. However, some dietary supplements may damage its epithelial lining, disrupt B cell development and weakening immune responses. Histological damage to epithelial cells can impair germinal center formation and antibody production (Oláh and Vervelde, 2009), underscoring the need for

supplement safety in poultry diets.

ACKNOWLEDGEMENT

Authors acknowledge College of Veterinary Medicine, University of Kerbala and Al-Furat Al-Awsat University, Technical College/Al-Musayyab for supporting this study.

NOVELTY STATEMENT

The study provides new insights into the revision of the feed supplementation to improve the performance of poultry and yielding higher productivity which can lead to security food vulnerabilities in the developing countries.

AUTHOR'S CONTRIBUTION

Shyaymaa Allawy Obed: Design and performed the experiments and data collection, analysis. Namir I. Mohammed: Methodology, writing review and editing the manuscript. Ali J. Jihad: Histological processing.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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