Effect of (Azolla Pinnata) Meal Supplementation on Growth Performance, Carcass Traits, and Gut Histomorphology in Commercial Broilers

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Tables 1 to 6 are available in the Supplementary Files section.

Graph 1 and 2 are available in the Supplementary Files section.

EFFECT OF (*AZOLLA PINNATA*) MEAL SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS TRAITS, AND GUT HISTOMORPHOLOGY IN COMMERCIAL BROILERS

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Abstract

This study explored Azolla pinnata meal as a sustainable alternative in broiler diets, using 300-day-old broilers. The four treatment groups (A, B, C, D) received 0g/kg, 1g/kg, 2g/kg, and 3g/kg of Azolla meal, respectively. Nutrient digestibility and nutritional analysis ensured a balanced diet. Results showed significant growth improvement, with the 3g/kg Azolla group achieving the most favorable feed conversion ratio (FCR) at 1.66, aligning with sustainability goals. A notable finding was the progressive increase in FCR over five weeks with week three displaying the highest FCR, is a unique finding in this study. Moreover, the observation of increasing FCR throughout the five-week experimental period, Higher Azolla supplementation correlated with increased ileal villus height, indicating improved gut health and nutrient absorption. Consistent crypt depth emphasized Azolla's specific impact on villus height. These results provide insights into Azolla pinnata meal's sustainable use in broiler nutrition, promoting growth, feed efficiency, and gut health. In conclusion, 3g/kg Azolla supplementation positively impacted broiler performance, emphasizing its potential in sustainable poultry production.

Keywords: *Azolla Pinnata* meal*,* feed intake, FCR, carcass traits, gut histomorphology, broiler.

Introduction

The global population will escalate to approximately 9.6 billion individuals by 2050. This substantial increase necessitates a parallel augmentation in food supplies, with a specific emphasis on dietary proteins. Within the realm of dietary proteins, the insufficiency of animal-derived proteins is a recurrent issue. Addressing this deficiency and bolstering the availability of animal proteins in both human and animal food supplies is a paramount concern for specialists in human and animal nutrition (Gibson and Jung, 2006). The poultry sector emerges as a vital player in this scenario, assuming a critical role in ensuring an adequate supply of animal protein. In light of the 2020 report from the Food and Agriculture Organization (FAO), there was an anticipated expansion in poultry meat production, projected to reach 137 million tons—a 2.6% increase in comparison to the figures recorded in 2019 ((FAO), 2020). Facing a significant global challenge, the poultry industry grapples with elevated feed costs, which alone constitute about 70%–80% of the total production expenses in feed formulation systems, predominantly relying on corn and soybean as the primary protein sources in poultry diets (Mwesigwa, 2021). Consequently, nutritionists are actively seeking ways to curtail feed ingredient expenses, exploring the utilization of by-products from diverse origins and unconventional food items. Spurred by this trend, researchers are investigating unconventional and alternative food sources, leading to the integration of Azolla into broiler chicken diets as a strategic approach to alleviate the significant feed expenses that account for nearly 70% of the total production costs.

Azolla is not only high in proteins, fatty acids, amino acids and vitamins (ref: https://www.sciencedirect.com/science/article/pii/S2352554121002084), but cost-effectiveness and adaptability in both wild and controlled farming environments (Saikia et al., 2014). It emerges as a valuable nutritional resource for various species, including poultry, livestock, and aquatic organisms. Previous research demonstrated that it could improve meat quality with enhanced colour, flavour, tenderness, and juiciness (Joysowal, 2018; Al-Rekabi et al., 2020).

Incorporating up to 5% air-dried Azolla in broiler chicken diets is beneficial in reducing final feed cost (which constitutes nearly 70% of total production expenses) for the farmers and, therefore, effective in managing substantial feed costs (Al-Rekabi et al., 2020). Basak et al. (2002) established Azolla spp.'s robust nutritional profile, suitable as a component in broiler feed rations, providing vital amino acids like leucine and alanine, as well as essential minerals such as iron, calcium, and magnesium. Rich in chlorophyll a, chlorophyll b, and carotenoid compounds (Mudgal et al., 2022; Lejeune et al., 2000), Azolla spp. Contains linolenic acid (9.8% to 37.95%) and linoleic acid (5.11% to 15.38%), surpassing some edible oils in linolenic acid content, showcasing its nutritional superiority (Lejeune et al., 2000).

Studies by Rengma et al. (2019), Shambhvi et al. (2021), and Rana et al. (2017) confirm Azolla spp.'s minimal aflatoxin content (0.01 microgram/kg), making it suitable for animal consumption without adverse health effects.

Saikia et al. (2014) determined the optimal dose, finding a 5% Azolla inclusion yielded final growth and efficiency comparable to the control group, making it economically viable, while a 15% inclusion led to diminished final growth. Azolla supplementation at a 2.5% level has been validated to enhance blood biochemical characteristics and positively affect broiler gizzard weight and breast muscle yield (Al-Rekabi et al., 2020; Shambhvi et al., 2021).

However, information regarding the impact of these dosage levels on gut histomorphology, a critical aspect for nutrient absorption and overall gut health, remains lacking. Addressing this gap, the current study scrutinizes the effects of Azolla pinnata meal supplementation on gut histomorphology and meat carcasses, aiming to enhance nutrient absorption while promoting sustainable and locally sourced alternatives for a resilient future in the poultry production industry.

2) Materials and Method

2.1 Study area, collection, and processing of (AZOLLA PINNATA) meal supplementation

The experiment was conducted at the Department of Poultry Science, and sample analysis was performed at the pathology laboratory of the College of Veterinary Science, Faculty of Animal Husbandry and Veterinary Science, the University of Agriculture, Peshawar.

Azolla was gathered as a green plant from agricultural banks and shallow water areas, dried in the sun right away and pulverized after complete drying. Dried Azolla (DA) sample was chemically analyzed before incorporating it in the broiler diet. The nutritional constituents of Azolla spp., including dry matter (DM), moisture, crude protein (CP), crude fibre (CF), and ether extract (EE), were evaluated following the methods stipulated in AOAC (2007) – specifically, 934.01, 976.05, 942.05, and 920.39, respectively. Additionally, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were analyzed following the methodology outlined by Goering and Van Soest (1970).

2.2 Experimental design and diets

Three-hundred-day-old chicks were individually weighed and randomly assigned to four treatments (A, B, C, and D). All chicks were weighed with a brand scale "CAMRY", 4800g range and 0.1g accuracy before being transferred, where they were divided into five replicates, with twelve birds in each replicate. Starter feed (as listed in Table 1) was provided during days 0 to 19, and finisher feed (as outlined in Table 2) was given from days 20 to 36. Diet formulations were calculated using FeedLIVE software version 1.52. The nutrient digestibility is presented in Tables 1 and 2, formulated based on Rathod et al. (2013) to meet chick requirements. The experimental basal diet was made to meet the requirements of chicks. Feed and water were provided ad libitum from days 1 to 35, with continuous lighting for twenty-four hours (with the aim to optimize feeding efficiency, reduce aggression, and promote continuous growth for the broilers). The experiment lasted five weeks, maintaining biosecurity to prevent disease spread and adjusting temperature and lighting according to environmental conditions.

2.3 Data collection

Daily and weekly feed intake, body weight gain (BWG), nutrient digestibility and feed conversion ratio (FCR) were calculated (Ullah et al. 2023). Daily mortality was recorded, and post-mortem examinations of dead birds were conducted. The dressing percentage was calculated with the help of the formula:

$$Dressed\ weight\ (\%) = \frac{dressed\ weight}{live\ weight} \times 100$$

Two birds from each treatment were collected at five weeks for feces collection and digestibility testing. Fresh morning feces were collected, weighed, dried for three days, and then analyzed. Nutrient digestibility was determined using the formula:

$$\text{Apparent nutrient digestibility (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

For gut morphometry, a randomly selected bird from each replicated group was slaughtered on the last day of the experiment. A small piece from the ileum portion of the small intestine was used for morphological measurements (Ullah et al. 2023). Economic parameters were determined by calculating input and output costs.

Giblet Ratio

Liver, heart and gizzard weights were measured and represented as a percentage of the total body weight.

2.3 Statistical Analysis

The data obtained were entered into Microsoft Excel Worksheets for grouping and basic statistics. The SAS general linear model (GLM) method and analysis of variance (ANOVA) were employed for data evaluation. The least significant difference (LSD) test was used to distinguish means, with the significance of means investigated at a 0.05 level of probability.

3. RESULTS

3.1. **The nutritional composition of Azolla pinnata during experiment is indicated in the table 3.**

The proximate analysis of Azolla pinnata revealed a well-balanced nutritional composition, with a moisture content of 94%, a notable concentration of crude protein at 23%, and a harmonious distribution of crude fat, crude fiber, ash, and nitrogen-free extract. In comparing Azolla pinnata with recognized nutritional standards, it becomes evident that it harmonizes well with recommended values for essential nutrients. This therefore, demonstrated its potential as a valuable dietary ingredient.

3.2. **Effect of Azolla pinnata meal supplementation in feed intake (g), weight gain and feed conversion ratio in broilers**

Table 4 provides a comprehensive overview of the impact of Azolla pinnata meal (APM) supplementation on feed intake, weight gain, and feed conversion ratio (FCR) in broiler chickens. Treatments include the control (Group A) with no APM supplementation, and Groups B, C, and D with increasing APM levels at 1g, 2g, and 3g per kg of feed, respectively.

A discernible trend in feed intake reveals Group B consuming the most feed at 3083g, followed closely by Groups A (3078g), with Group D exhibiting the lowest intake at 3072g, displaying no statistically significant differences.

In terms of weight gain, a positive correlation is observed with increasing APM supplementation, with Group D leading at 1816.0g. Notably, despite APM supplementation, Group B shows the lowest weight gain at 1765.0g.

Regarding FCR, the study indicates a decrease with higher APM levels, with Group D achieving the lowest FCR at 1.66, signifying improved feed utilization. Figure 1 illustrates FCR over the five-week period, with week three showing the highest FCR (compare to other time periods). Groups C and D consistently outperform the control, emphasizing the benefits of APM supplementation.

3.3. **Effect of Azolla pinnata meal supplementation on Gut Histomorphology**

This study evaluates the impact of varying APM levels on gut histomorphology, examining parameters like Villus Height (VH), Crypt Depth (CD), Villus Width (VD), and the Villus-Crypt Ratio (VCR) (Table 5 and Figure 2).

The VCR is significant in evaluating the health and functionality of the small intestine. A heightened VCR typically signifies improved nutrient absorption and overall gut health, attributed to taller villi and shallower crypts, resulting in an expanded surface area for nutrient absorption.

Data revealed a substantial increase ($P<0.05$) in VH with increasing levels of Azolla supplementation. This pattern is evident as Group D showcases the most significant villus height at 0.91, surpassing Groups C (0.70), B (0.66), and the control group (0.48). Intriguingly, the CD remained consistent across the groups, displaying no significant variations.

Focusing on VCR, Group D once again stands out, achieving the highest VCR at 3.93, indicative of a healthier gut environment. Following closely is Group B, whereas the control group (Group A) registers the lowest VCR in this study.

3.4 Effect of Azolla pinnata meal supplementation on Dressing Percentage and Giblet Weight

Graph 1 indicates a noteworthy enhancement ($P<0.05$) in dressed body weight percentage with increasing APM levels. Group C achieves the highest dressing percentage at 71%, followed by Group B (2g/kg APM) at 68.00% and the control group at 67.00%, while Group A (1g/kg APM) trails with the lowest dressing percentage at 66.00%. This highlights a positive correlation between APM supplementation and dressed body weight percentage.

Contrastingly, Graph 2 shows no significant difference ($P<0.05$) in giblet weight across treatments, suggesting that APM supplementation does not substantially alter giblet weight in broilers.

3.5. **Effect of Azolla pinnata meal supplementation on economics parameter**

Table 6 indicates the economic implications of different APM levels in broiler feed, focusing on feed expenses, gross return, and net return per chick.

Higher APM supplementation correlates with increased total feed costs leading to a substantial increase from PKR 445.49 in the control group (Group A) to PKR 557.47 in Group D (3g APM/kg feed). However, this rise is offset by positive trends in both gross and net returns, particularly evident in Groups C and D. Group D stands out with an impressive mean net return of PKR 54.42, emphasizing the potential financial gains through increased APM supplementation.

4. Discussion

In this investigation, the effects of varying levels of APM supplementation on broiler performance were comprehensively examined, focusing on feed intake, weight gain, and feed conversion ratio. The study consistently demonstrated that Group D, receiving the highest APM supplementation (3g per kg), exhibited the most favorable outcomes, emphasizing the potential benefits of APM at this level. Notably, lower APM levels (groups A, B, and C) did not significantly impact feed efficiency. An intriguing observation emerged regarding the FCR trends over the five-week period, with week three standing out as having the highest FCR among all weeks. This unique pattern suggests a potential shift in feed efficiency during the third week, warranting further exploration to unveil insights into optimizing feeding strategies during specific growth stages.

The high protein content, essential amino acids, and minerals in Azolla spp. likely contributed to the improved weight gain in broiler chickens. Additionally, the increased metabolizable energy in diets containing higher APM levels may have influenced APM digestibility, enhancing feed conversion efficiency in Group D. These findings align with previous studies by Joysowal et al. (2018), Chatterjee et al. (2020), Kamel and Hamed (2021), all reporting improved feed efficiency in broilers fed with Azolla. However, discrepancies in results across studies, noted by Rawat et al. (2015), Abdelatty et al. (2021) and Samad et al. (2020), emphasize the influence of broiler strains, environmental factors, and varied supplementation doses on outcomes.

The final body weight of broilers in Group D, fed with 3g of Azolla, demonstrated the highest weight gain, attributed to Azolla's enriched nutrient profile. Mineral elements highlighted in the study by Dhumal et al. (2009) and the antioxidant properties of carotenoids and biopolymers, as noted by Acharya et al. (2015), likely contributed to the observed benefits.

While these findings are consistent with some studies, including Samad et al. (2020) and Ara et al. (2015), showing increased body weight gain with Azolla supplementation, discrepancies with Abdelatty et al. (2020), Paudel et al. (2015), and Sujatha et al. (2013) suggest potential variability influenced by supplementation levels, broiler age, and feed type.

The investigation into gut histomorphology revealed significant improvements, particularly in Villus Height and the Villus-Crypt Ratio, indicating enhanced nutrient absorption and overall gut health. The observed correlation between increased Azolla levels and improved weight gain supports the positive impact of APM on broiler gut health. These results align with Abdelatty et al. (2021), who noted a significant effect of Azolla meal on broiler intestinal morphology. However, contrasts with Ara et al. (2022) suggest some variation across studies, emphasizing the need for further research.

Concerning giblet weight, no significant effects were observed in broilers fed with Azolla-supplemented diets compared to the control group. This consistency with established theories underlines the importance of amino acids in poultry diets and suggests that Azolla can be integrated without adverse effects on giblet development, highlighting its potential as a sustainable feed ingredient. These outcomes align with previous research by Yadav et al. (2014), Abdelatty et al. (2021), and Naghshi et al. (2014), emphasizing the lack of significant impact on giblet percentage with Azolla pinnata meal. Additionally, the variations in dressing percentage among treatments, reported by Shambhvi et al. (2021), underscore the complexity of dietary effects on broiler physiology.

In summary, this study sheds light on the multifaceted impacts of Azolla pinnata meal supplementation on broiler performance, gut health, and economic parameters. The consistent trends observed, alongside variations in certain aspects, provide valuable insights for further exploration and optimization of Azolla inclusion in broiler diets.

CONCLUSION AND RECOMMENDATION

In summary, the current study has revealed significant advancements in broiler performance and gut health attributed to the inclusion of Azolla meal in their diets. Notably, the addition of 3g/kg of Azolla meal had a profound impact on several key parameters. This study demonstrated that supplementing with 3g/kg of Azolla meal significantly enhanced essential aspects of broiler growth performance, including feed intake, weight gain, and dressing percentage. The most compelling discovery was the remarkable improvement in feed conversion ratio (FCR) achieved by the group receiving the highest Azolla supplementation level, underscoring the critical importance of fine-tuning dietary formulations for optimal feed efficiency in broiler production.

Furthermore, our research shed light on the profound effects of Azolla meal on gut histomorphology, particularly the substantial increase in ileal villus height. This positive alteration in gut structure signifies enhanced nutrient absorption capacity, potentially contributing to the improved growth observed in the study.

Therefore, it is imperative that further research ventures address the long-term effects of Azolla supplementation on broiler performance and gut health, investigate optimal inclusion levels for diverse broiler breeds and production systems, and elucidate the underlying mechanisms responsible for the favorable effects of Azolla pinnata meal on broiler growth and gut histomorphology.

5. ACKNOWLEDGEMENTS

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Animal welfare statement

This study was approved by the animal welfare and care committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools were considered to minimize the pain and discomfort of birds during the conduction of this experiment.

IRB approval

The study was approved by Advanced Studies and Research Board (ASRB), (No.1145/ASRB/UAP, dated 08/05/2023).

Ethical statement

The experiment was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences (FAHVS), The University of Agriculture Peshawar before the practical execution of this experiment.

Authors Contribution

Faheem Ullah Khan, Rafiullah conceived the idea, performed experiment. Lionel Kinkpe performs data analysis and prepared the first draft of the manuscript. The final draft of the manuscript was rewritten, reviewed, and edited by Sohaib Ul Hassan, Hafiz Qadeer Ahmed, Lionel KINKPE, Muhammad Shuaib and Cedric Assogba. Rafiullah supervised overall research.

Data Availability

Data is available upon request

Disclosure statement

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

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Figures

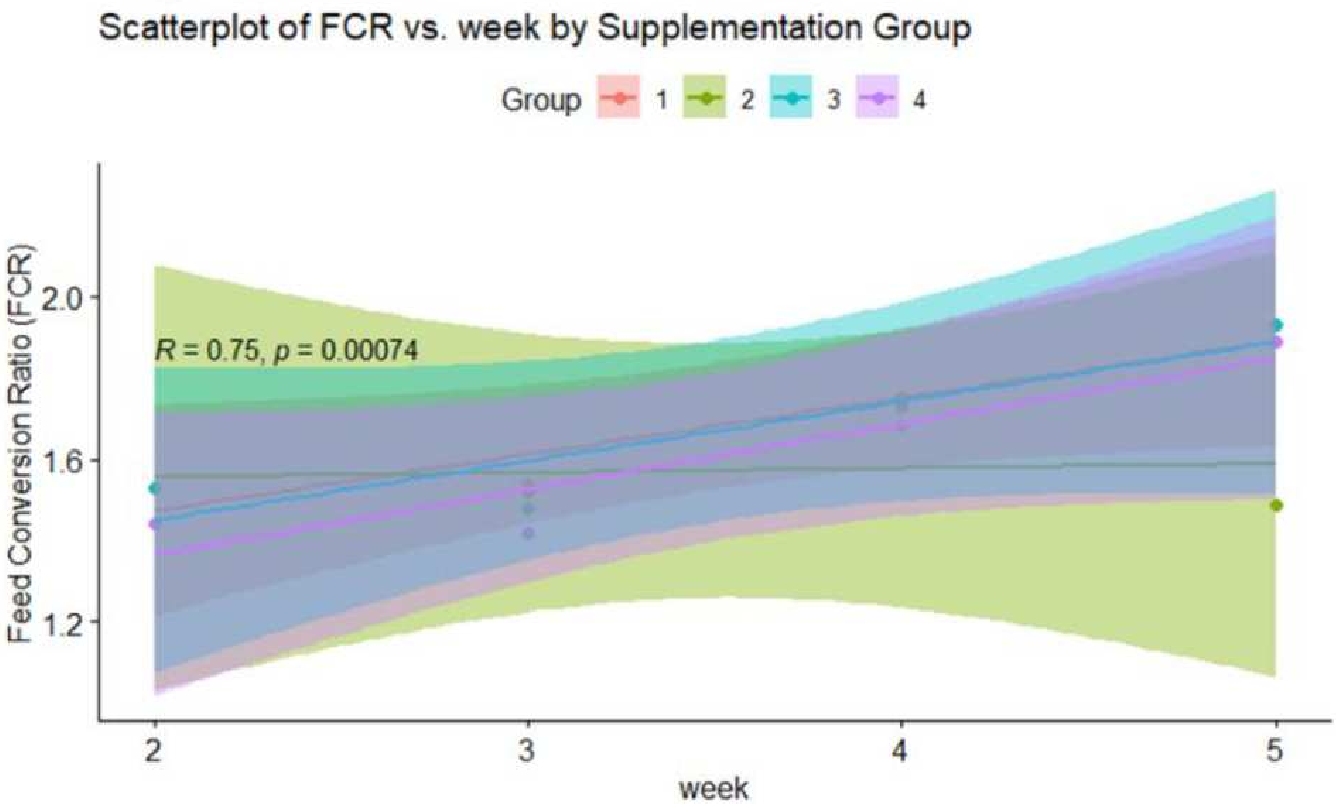

Figure 1

Scatterplot of FCR vs Week supplementation Group.

Group1=GroupA; Group2=GroupB; Group3=GroupC; Group4=GroupD

The scatter plot above illustrates the Feed Conversion Ratio (FCR) for different groups of broilers across five weeks. Each point on the plot represents the FCR for a specific group in a given week, with different colors indicating different groups.

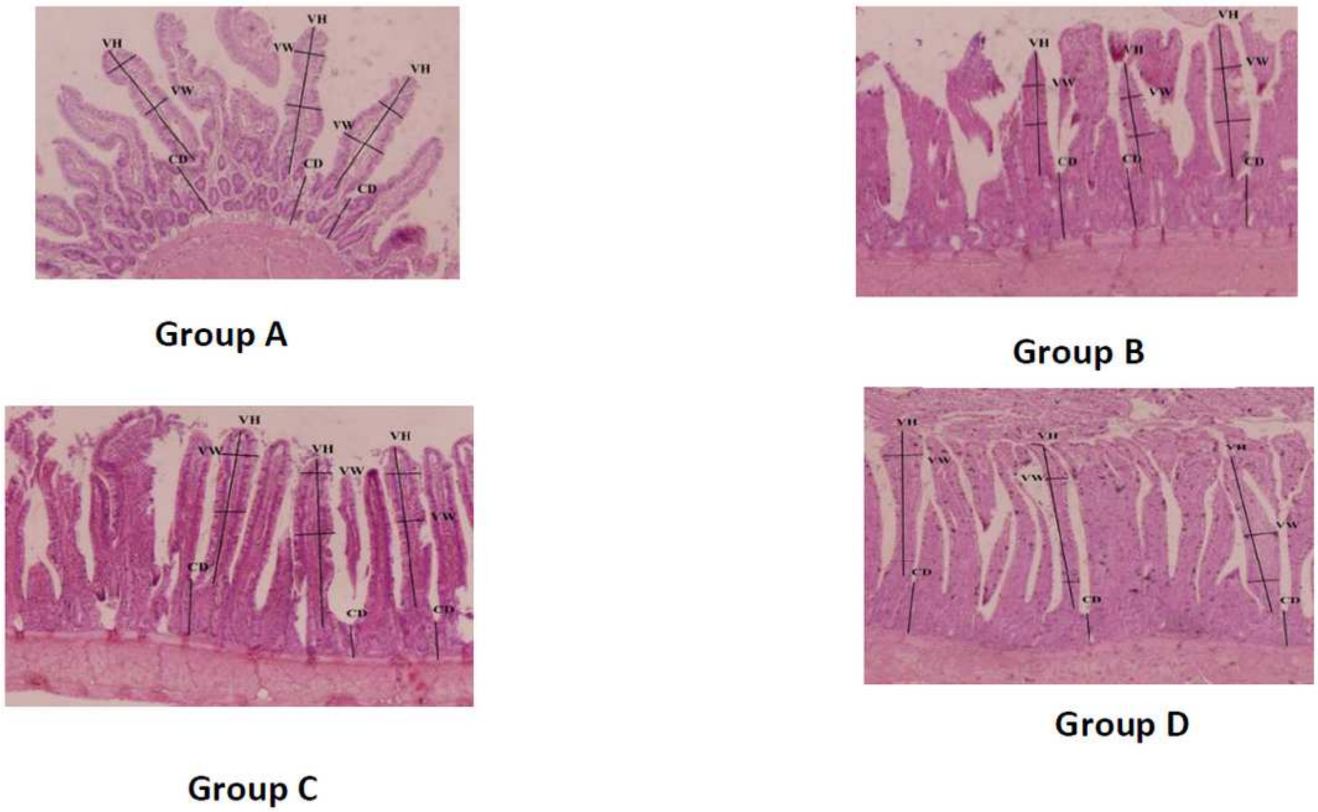

Figure 2

Histomicrograph 10X showing the effects of different levels Control, A = 1g APM/kg feed, B = 2g APM/kg feed, C = 3g APM/kg of azolla on the histological structure of the intestine of broilers.

VH: Villus height (length); VW: Villus width; CD: Crypt depth. The villus height, villus width and crypt depth are in millimeter

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.pdf
- G1.png
- G2.png