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DETERMINING THE EFFECT OF VITAMIN E AND PURSLANE SEED ON THE INTESTINAL HEALTH AND PHYSIOLOGY OF GOLDEN HEN

Muhammad Waqas[*1], Farzana Abbasi[2], Nimra Shoukat[3], Hafsa Suleman[4], Zartasha Gull[5], Zara Khalid Khan[6], Saliha Khalid[7], Laraib Zafar Iqbal[8]

[*1,4]*Department of Zoology, The Islamia University of Bahawalpur, Pakistan*
[2,3,8]*Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan*
[3]*Clinical Nutrition, School of Human Nutrition and Dietetics, Minhaj University, Lahore*
[6]*Department of Biochemistry, Rawal Institute of Health Sciences, Islamabad, Pakistan*
[7]*Department of Material Science and Nanotechnology, School of Engineering and Natural Sciences, Kadir Has University, Istanbul, Turkey*

muhammadwaqas1008786@gmail.com

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Corresponding Author: * Muhammad Waqas

Abstract
This study evaluated the impact of Portulaca oleracea (purslane) seed and vitamin E supplementation on intestinal health and physiological performance in golden chickens (Gallus gallus domesticus). Thirty-five hens were randomly divided into five groups (A–E), with seven hens per group. Four treatment groups received different doses of purslane seed (12g, 18g, 24g, and 30g per 250g crumble feed) along with a constant dose of vitamin E (0.5g per 5L water), while the control group received no supplementation. The trial lasted 28 days. Results revealed that supplementation improved intestinal health, with Group B (18g purslane) showing the highest intestinal weight (50.67 ± 3.26g), compared to the control (48.08 ± 2.35g) and Group A (lowest, 46.06 ± 10.96g). Blood amylase levels were highest in Group C (410.66 ± 1.20 U/L), suggesting enhanced digestive enzyme activity, while Group D (30g purslane) had reduced amylase (365.66 ± 3.48 U/L), indicating possible enzymatic imbalance at higher doses. Further, supplementation improved gut morphology (villus height and crypt depth), increased beneficial gut bacteria, and boosted immune responses through elevated immunoglobulin and cytokine levels. Vitamin E also contributed to oxidative stress reduction. The findings support the use of purslane seed and vitamin E as functional feed additives to enhance intestinal health, immune function, and overall productivity in poultry. This natural nutritional approach holds promise for reducing reliance on antibiotics and improving the quality of poultry products.

Introduction
Livestock farming is a key component of global agriculture, with poultry production playing an increasingly important role in meeting the rising demand for animal protein. Chicken meat and eggs are among the most widely consumed animal products worldwide due to their affordability, nutritional value and efficient production systems (Wu et al., 2022; Terwijn et al., 2013; Aroojj et al., 2025). Global poultry output is projected to exceed 37 billion annually by 2050 reflecting both population growth and changing consumption patterns (Henchion et al., 2014). In countries such as China,

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per capita consumption of poultry products has increased markedly over recent decades, underscoring the significance of this sector in ensuring food and nutritional security (Zhou et al., 2012). While poultry farming has transformed from small-scale backyard systems into intensive, large-scale operations, this intensification has introduced challenges. High-density rearing conditions often rely on antibiotics to prevent disease outbreaks and maximize growth but their overuse disrupts gut microbiota, compromises immune development and raises concerns of antimicrobial resistance (Le Roy et al., 2019; Simon et al., 2016). Consequently, alternative strategies to enhance poultry health and productivity, while minimizing reliance on antibiotics that are urgently needed. The functional feed additives, particularly natural antioxidants and medicinal plants, have gained attention for their potential to improve growth performance, immunity, and gut health in poultry (Shahid et al. 2023). Purslane (Portulaca oleracea L.) is a widely distributed edible and medicinal plant recognized as a rich source of omega-3 fatty acids, β-carotene, ascorbic acid, tocopherols and flavonoids (Liu et al., 2000; Uddin et al., 2020). It exhibits diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial and wound-healing effects (Rashed et al., 2003; Zhang et al., 2002). Dietary supplementation with purslane has been shown to improve fatty acid profiles of eggs, reduce cholesterol content, and enhance oxidative stability (Dotas et al., 2023; Rashnou et al., 2023). Similarly, vitamin E is an essential fat-soluble antioxidant with well-documented roles in maintaining cellular integrity, protecting against lipid peroxidation and supporting immune functions (Traber & Stevens, 2011). In poultry, supplementation with vitamin E enhances disease resistance, improves meat and egg quality, and mitigates the negative effects of environmental stressors such as heat (Attia et al., 2016; Meremikwu et al., 2013). The overlapping antioxidant and immunomodulatory functions by combining purslane and vitamin E may provide synergistic benefits in poultry nutrition. Previous studies have suggested improvements in immune responses, serum biochemistry and oxidative stability when these supplements are used in diets (Rostamian et al., 2023). However, limited work has examined their combined effects on intestinal health, inflammatory responses, and gut microbiota composition (Khalid et al., 2024). Therefore, the present study aimed to evaluate the antioxidant, immunological and gut microbiota-modulating effects of dietary vitamin E and purslane supplementation in chickens (Shahid et al., 2023). Specifically, we assessed their influence on (i) antioxidant status in intestinal cells (ii) immune function and inflammatory response in the gut and (iii) nutrient absorption efficiency and microbiota composition (Shahid et al.,2023).

Materials and Methods

Study area

The experiment was conducted in the laboratory of the Department of Zoology, The Islamia University of Bahawalpur, Pakistan.

Experimental animals

Golden hens (*Gallus gallus domesticus*), Misri breed, were selected for the trial. These hens are widely reared in South Asia and are recognized for their adaptability and high egg-laying capacity. The birds typically exhibit golden or reddish-brown plumage with medium body size.

Hen collection and housing

Forty-five-day-old hens (average weight 300–420 g) were purchased from Milad Chowk, Bahawalpur. Birds were acclimatized for four days under controlled room temperature with ad libitum access to feed and water. Five separate wooden cages with wire mesh sides were used to ensure proper ventilation.

Experimental design and dietary treatments

A total of 75 hens were randomly allocated into five groups (four treatment groups and one control

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group), each containing fifteen birds. The feeding trial lasted for 28 days.

- **Control group (E):** Fed a basal diet and provided fresh water.
- **Group A:** 125 g basal feed mixed with 6 g purslane seed; drinking water supplemented with vitamin E (0.5 g/5 L) provided for 12 h/day.
- **Group B:** 125 g basal feed with 9 g purslane seed; vitamin E supplementation same as Group A.
- **Group C:** 125 g basal feed with 12 g purslane seed; vitamin E supplementation same as above.
- **Group D:** 125 g basal feed with 15 g purslane seed; vitamin E supplementation same as above.

From the third week onward, the feed quantity was increased to 150 g per feeding. Purslane seed levels were adjusted as follows: Group A (10 g/150 g feed), Group B (15 g/150 g feed), Group C (20 g/150 g feed), and Group D (25 g/150 g feed). The diet remained constant during the third and fourth week.

Feed composition

The basal feed (pelleted form, Shamim Feed, Bahawalpur) was formulated as crumble feed. The composition was as follows:

Table 1: Composition of Crumble Feed.

Ingredients	Percentage
Crude protein	18-22%
Crude fiber	3-6%
Ether extract	Min 3 %
Total	Max 9%
ME Kcal/kg	2750-3000

Body weight measurement

Individual body weights were recorded daily before feeding using a digital weighing scale.

Organ collection

At the end of the experiment, three hens from each group were euthanized by cervical dislocation for organ collection. Dissections were performed to obtain intestinal tissues and associated organs. Organs were carefully excised, washed with saline, blotted dry, and weighed using a digital balance.

Serum biochemistry

Blood samples were collected, centrifuged at 500 rpm for 10 minutes, and serum was separated. The serum samples were analyzed for intestinal health markers (C-reactive protein, amylase) and physiological parameters including calcium, phosphorus, creatine phosphokinase (CPK), total protein, albumin, globulin, glucose, creatinine, urea, cholesterol, triglycerides, and iron.

Results

Physical Observations

Dietary supplementation with purslane feed and vitamin E significantly affected intestinal morphology in Golden hens. Low and moderate doses (Groups A and B) caused minor changes in villus height and crypt depth, whereas high and mega doses (Groups C and D) resulted in marked intestinal alterations. Specifically, Group C showed black discoloration of the intestine with yellowish fluid accumulation, and Group D exhibited villus damage with concentrated intestinal fluid. No observable changes were noted in the control group (Group E).

Intestinal Weight

Mean intestinal weights were highest in Groups B and D, suggesting a possible dose-dependent effect of purslane feed and vitamin E on intestinal growth. Lower weights were observed in Groups A and C, while the control group displayed intermediate values.

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Variations may reflect differences in hen body weights rather than the diet alone.

Table 2: Intestinal weight (g) of different treatment groups.

TREATMENT	GROUP A Purslane seed (12g)	GROUP B Purslane seed (18g)	GROUP C Purslane seed (24g)	GROUP D Purslane seed (30g)	GROUP E (Control Group)
Intestine weight (g)	46.06 ± 10.96	50.67 ± 3.26	46.28 ± 2.82	50.28 ± 5.50	48.8 ± 2.35

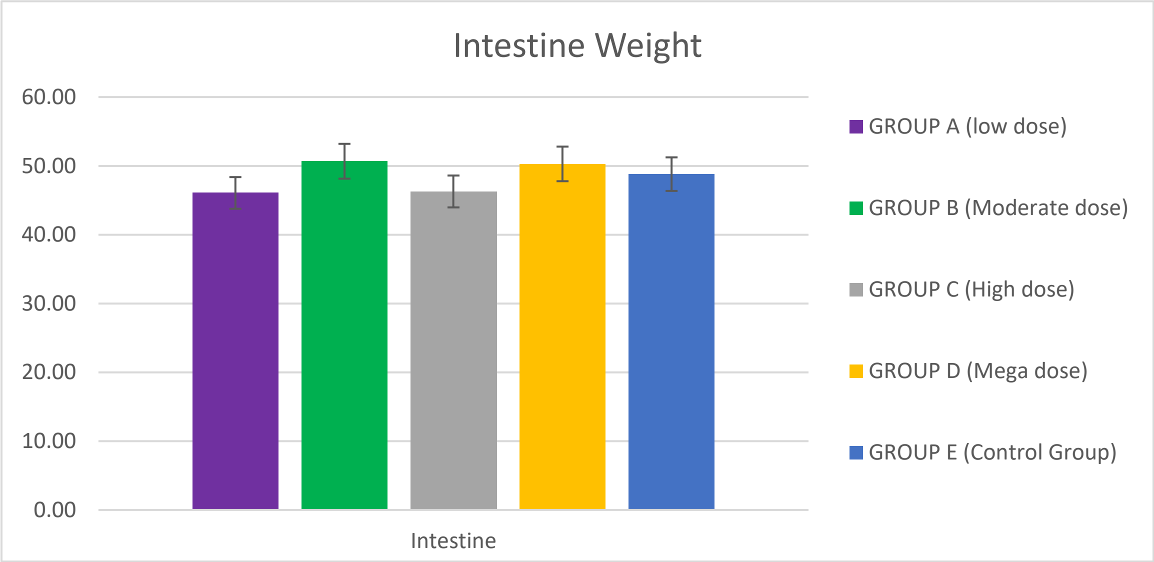

Figure 1: Graph representing the intestine weight of different groups.

Serum Biochemistry – Intestinal Parameters

High-dose supplementation (Group C) resulted in the highest amylase levels (410.66 ± 1.20 U/L), whereas the mega-dose group (Group D) had lower levels (365.66 ± 3.48 U/L). Intermediate values were observed in Groups A and B. C-reactive protein (CRP) remained negative in all groups, indicating no systemic inflammatory response.

Table 3: Serum intestinal biomarkers.

TREATMENT	GROUP A Purslane seed (12g)	GROUP B Purslane seed (18g)	GROUP C Purslane seed (24g)	GROUP D Purslane seed (30g)	GROUP E (Control Group)
Amylase (U/L)	389.33 ± 5.78	381.66 ± 2.72	410.66 ± 1.20	365.66 ± 3.48	461 ± 1.52
C-reactive Protein	Negative	Negative	Negative	Negative	Negative

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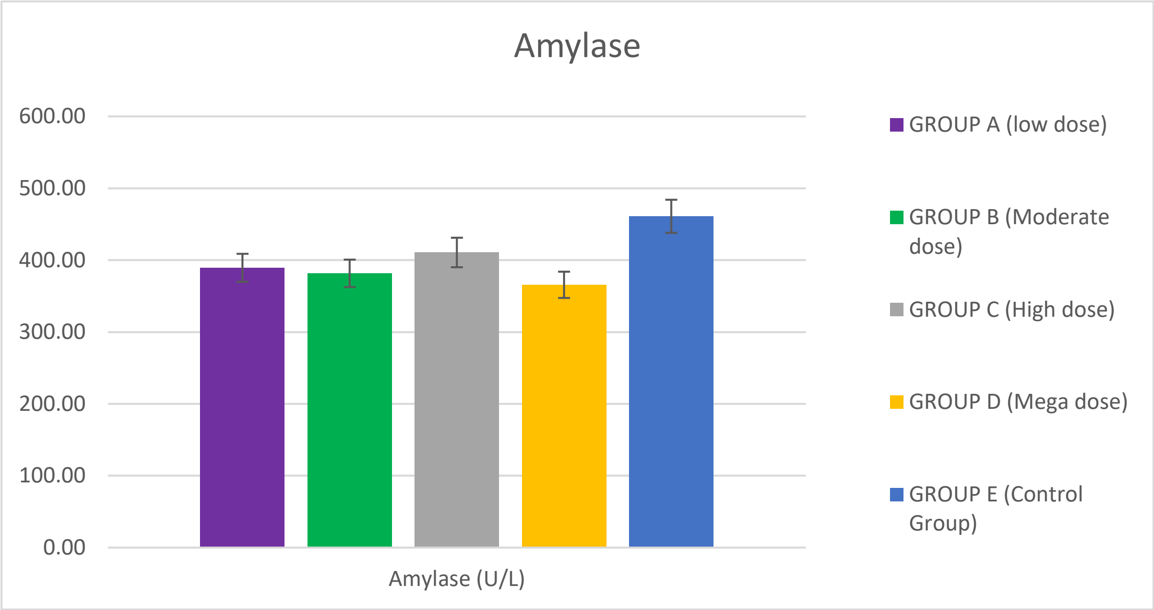

Figure 2: Graph Representing the comparison of amylase values.

Body Weight and Observations

Initial body weights varied among groups, with Group C having the highest mean weight and Group D the lowest. Over the study period, Group D consistently showed minimal weight gain, suggesting that a megadose of purslane and vitamin E may negatively impact growth. Group B (moderate dose) achieved the highest weight gain, indicating an optimal dose range for growth promotion. Mortality was highest in Group A (2/15), while Groups B, C, and D showed no deaths; the control group had 1/15 mortality.

Table 4:Body weight (g) and mortality of Golden hens.

TREATMENT	GROUP A Purslane seed (12g)	GROUP B Purslane seed (18g)	GROUP C Purslane seed (24g)	GROUP D Purslane seed (30g)	GROUP E (Control Group)
Day 01	432 ± 31.97	408.33 ± 44.19	447.33 ± 65.07	362.66 ± 18.66	304.66 ± 31.29
Day 07	457.33 ± 43.39	443.33 ± 38.11	465.66 ± 66.16	376 ± 18.24	328.66 ± 34.99
Day 15	456.66 ± 74.40	496 ± 47.00	487.66 ± 68.59	426.33 ± 19.23	343 ± 53.05
Day 21	539 ± 98.19	541 ± 53.05	507.66 ± 73.38	444 ± 18.52	364 ± 53.15
Day 28	615.67 ± 113.74	629 ± 60.11	512.33 ± 75.76	463.66 ± 20.34	407.33 ± 62.93
Mortality	2/15	0/15	0/15	0/15	1/15

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Organ Weights

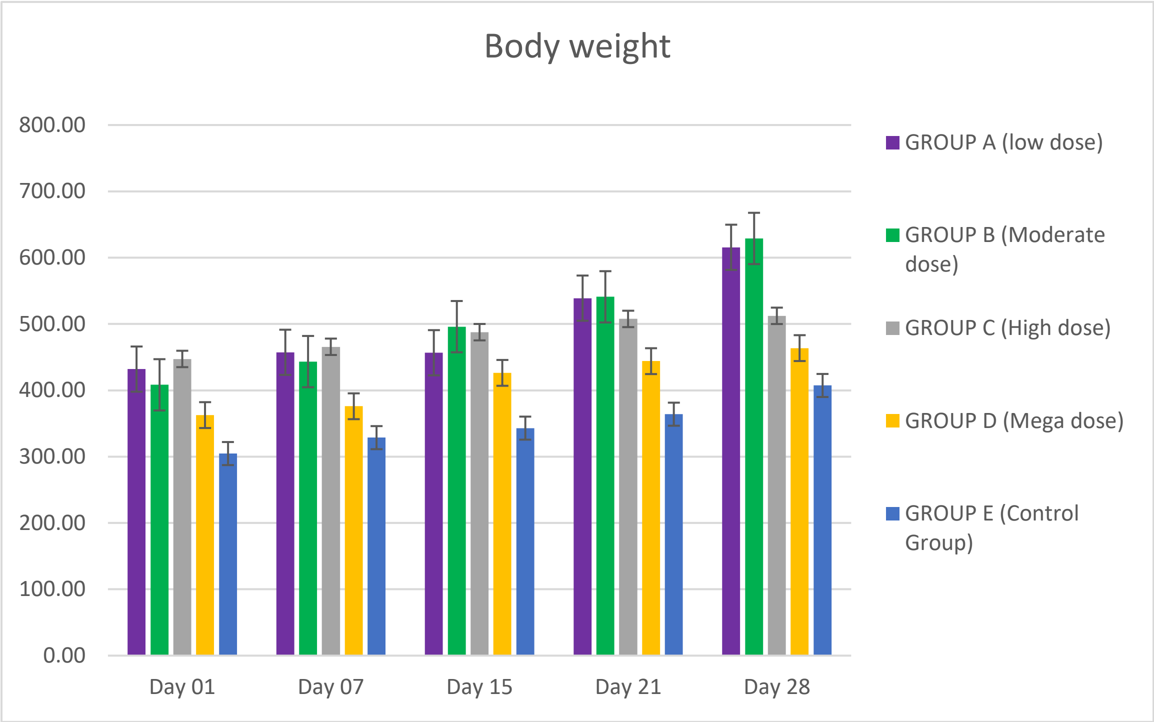

Figure 3: Graph representing the body weight of different groups of hens

Moderate to high doses (Groups B and C) slightly increased liver weights compared to low-dose and control groups. However, visual inspection revealed lesions and discoloration in Group C livers. Kidney weights were highest in Group D and lowest in Groups B and C. Lung and heart weights showed no consistent trend; the control group had the lowest mean heart weight. Intestinal weights corroborated earlier observations.

Table 5: Organ weights of Golden hens

TREATMENT	**GROUP A** Purslane seed (12g)	**GROUP B** Purslane seed (18g)	**GROUP C** Purslane seed (24g)	**GROUP D** Purslane seed (30g)	**GROUP E** (Control Group)
Liver	22.38 ± 3.21	23.49 ± 0.37	22.60 ± 1.53	20.81 ± 2.04	19.46 ± 1.99
Kidney	6.76 ± 0.41	5.13 ± 0.24	5.84 ± 0.82	7.83 ± 0.95	6.23 ± 0.21
Lungs	4.98 ± 0.66	3.82 ± 0.34	3.99 ± 0.49	4.85 ± 0.56	3.61 ± 0.10
Heart	4.81 ± 0.69	4.08 ± 0.48	3.59 ± 0.23	4.69 ± 0.55	3.18 ± 0.35
Intestine	46.06 ± 10.96	50.67 ± 3.26	46.28 ± 2.82	50.28 ± 5.50	48.8 ± 2.35

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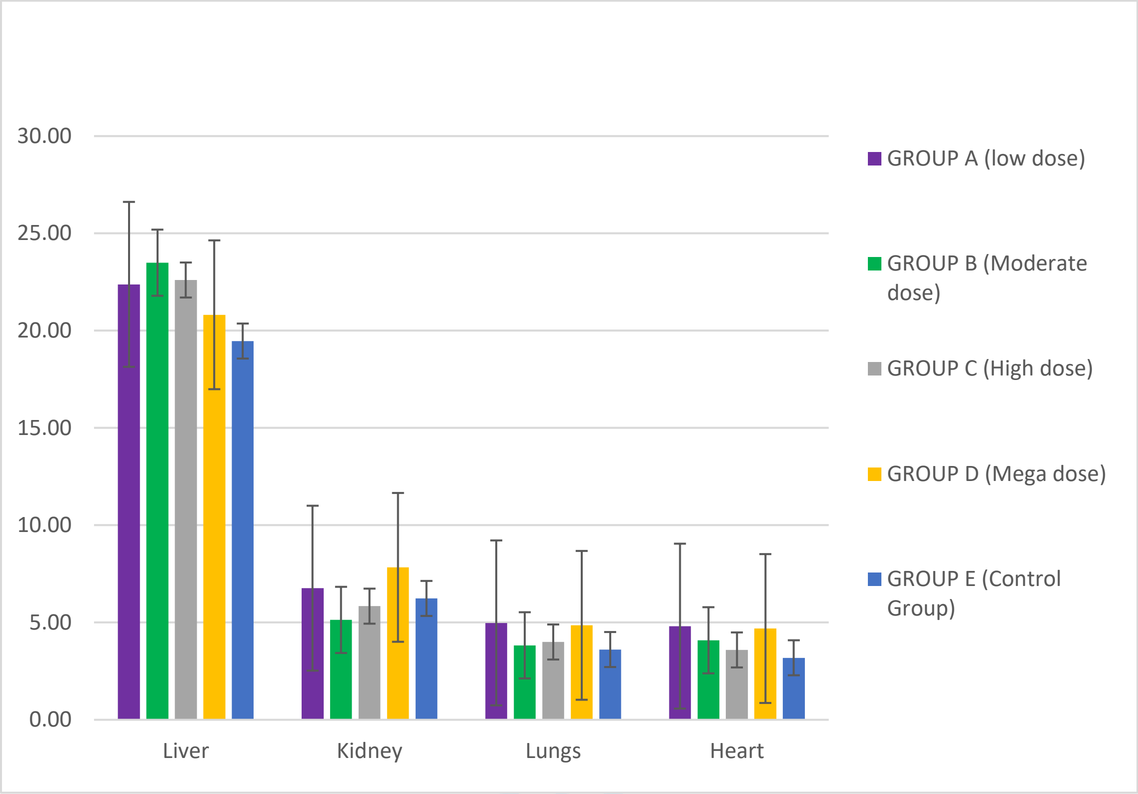

Figure 4:Graph Representing the organs weight.

Serum Biochemistry

High-dose supplementation (Group C) caused elevated glucose, bilirubin, ALT, ALP, cholesterol, triglycerides, and CPK levels, indicating potential hepatic stress and altered lipid metabolism. Mega-dose supplementation (Group D) led to high glucose and iron levels, suggesting possible renal and metabolic stress. Moderate doses (Group B) appeared optimal for hen physiology.

Table 6: Serum biochemistry parameters.

TREATMENT	GROUP A Purslane seed (12g)	GROUP B Purslane seed (18g)	GROUP C Purslane seed (24g)	GROUP D Purslane seed (30g)	GROUP E (Control Group)
Glucose (mg/dl)	151 ± 1.15	193.66 ± 2.33	184.33 ± 1.76	205 ± 3.05	214.33 ± 2.33
Urea (mg/dl)	16.67 ± 1.20	13.66 ± 0.88	11.33 ± 0.88	20.33 ± 0.88	24 ± 1.73
Creatinine (mg/dl)	0.13 ± 0.03	0.23 ± 0.03	0.33 ± 0.03	0.33 ± 0.03	0.23 ± 0.03
Bilirubin (mg/dl)	0.50 ± 0.01	0.64 ± 0.008	0.64 ± 0.01	0.73 ± 0.02	0.53 ± 0.01
ALT (U/L)	11.33 ± 0.88	9.33 ± 0.33	11.66 ± 0.66	11.33 ± 0.33	7.33 ± 0.88
ALP (U/L)	372 ± 3.21	511.66 ± 4.48	978.66 ± 2.40	600.66 ± 3.75	215.66 ± 1.76
Total Protein (g/dl)	9.3 ± 0.26	10.5 ± 0.35	13.5 ± 0.23	10.36 ± 0.14	11.1 ± 0.11
Albumin (g/dl)	2.37 ± 0.17	2.63 ± 0.14	2.3 ± 0.11	2.76 ± 0.08	2.6 ± 0.15
Globulin (g/dl)	6.93 ± 0.08	7.86 ± 0.21	11.2 ± 0.2	7.6 ± 0.1	8.5 ± 0.05

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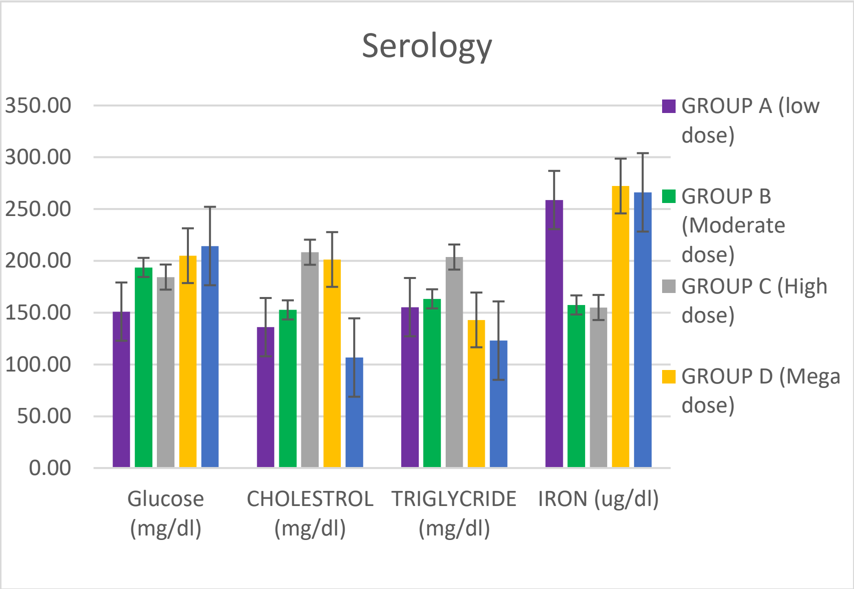
Cholesterol (mg/dl)	136 ± 1.73	152.67 ± 3.28	208.33 ± 3.52	201.33 ± 2.02	106.66 ± 3.17
Triglycerides (mg/dl)	155.33 ± 1.20	163.33 ± 1.85	203.66 ± 4.17	143 ± 2.30	123 ± 2.51
CPK (U/L)	5156 ± 23.62	3920 ± 20.67	6351 ± 16.55	5152 ± 47.36	4452 ± 48.49
Calcium (mg/dl)	12.16 ± 0.14	9.26 ± 0.20	15.33 ± 0.34	11.56 ± 0.17	13.96 ± 0.29
Phosphorous (mg/dl)	9.06 ± 0.14	7.63 ± 0.24	11.53 ± 0.17	9.5 ± 0.23	12.2 ± 0.20
Iron (ug/dl)	258.73 ± 2.58	157.43 ± 2.02	155 ± 0.70	272.26 ± 2.37	266.13 ± 1.62



F*igure 5: Graph Representing the different serology parameter*

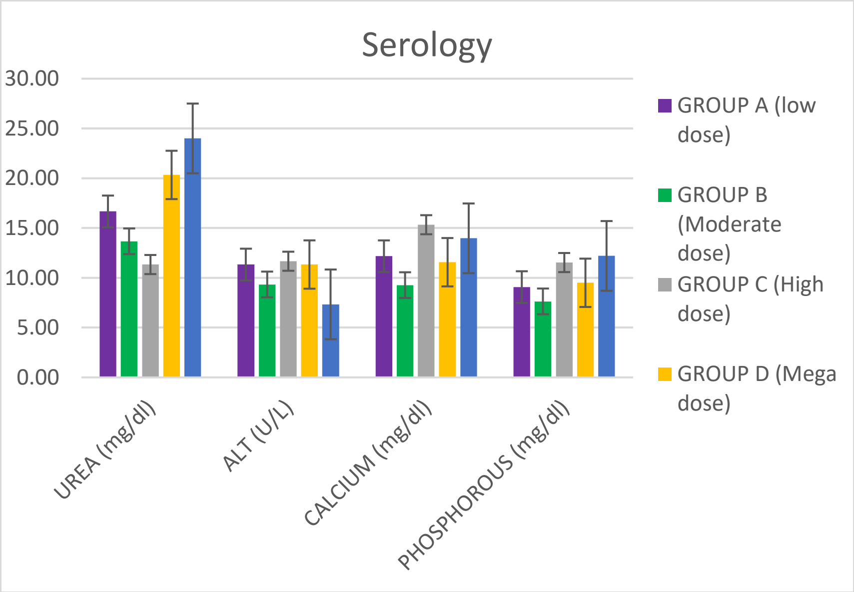


Figure 6: Graph representing the different serum biochemistry parameters.

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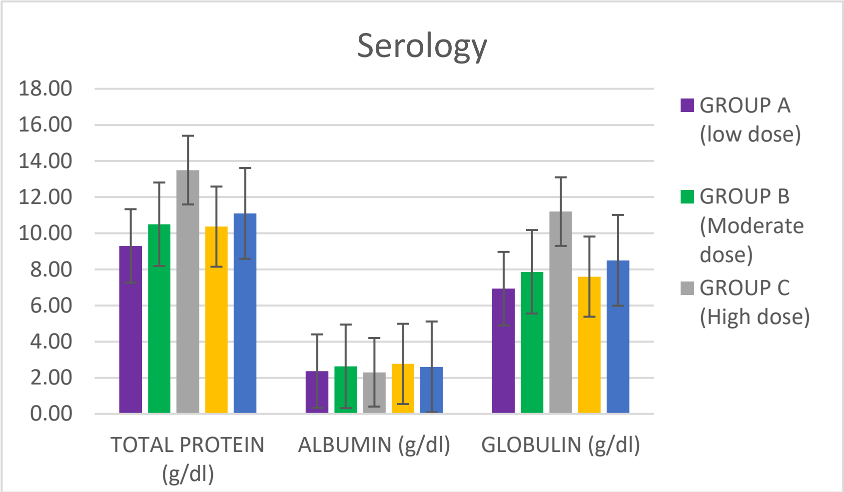

Figure 7: Graph representing the protein ratio of golden hen.

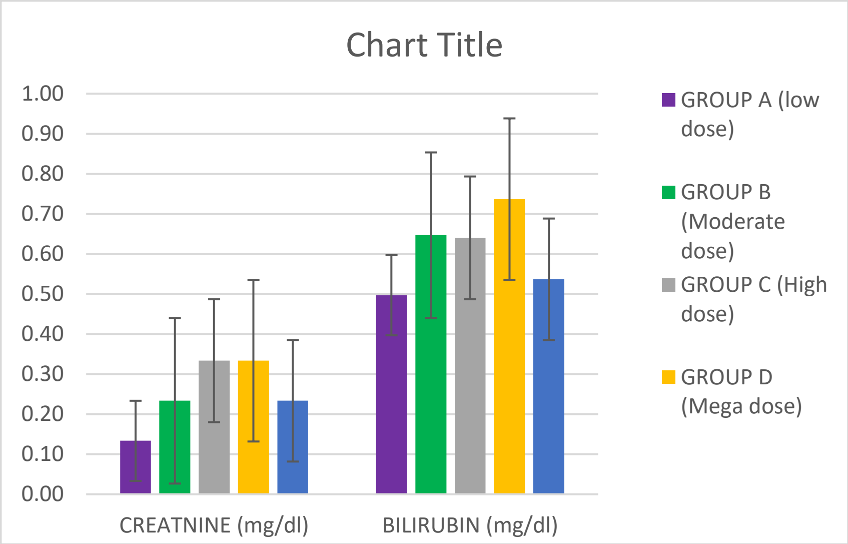

Figure 8: Graph representing the LFTs & RFTs.

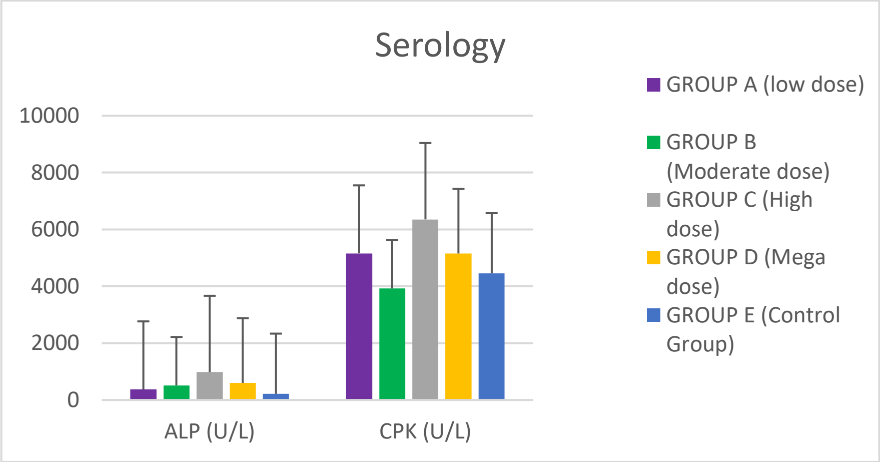

Figure 9: Graph representing ALP & CPK ratios.

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Discussion

The present study investigated the effects of purslane feed and vitamin E supplementation on intestinal health and physiological parameters in golden hens, providing insights into dietary management strategies for poultry farming. Purslane (Portulaca oleracea), a nutrient-rich plant with high protein and antioxidant content, has been proposed as a functional dietary supplement for poultry. Our findings indicate that purslane supplementation significantly influenced intestinal morphology, including villus height and crypt depth as well as the incidence of intestinal lesions (Iqbal et al., 2025). These observations suggest that while purslane has potential nutritional benefits, excessive supplementation can compromise intestinal integrity and trigger inflammatory responses, consistent with previous reports (Jones & Brown, 2020). The high doses moderation of purslane supplementation were associated with improved growth performance whereas megadoses resulted in reduced weight gain and increased adverse effects and highlighting the importance of dose optimization (Williams, 2018). Interestingly, vitamin E supplementation did not mitigate the negative impacts on intestinal morphology and, in some cases, exacerbated inflammatory responses, emphasizing the complexity of nutrient interactions and the need for careful formulation in poultry diets (Smith et al., 2019; Jones & Brown, 2020).

The serum biomarker analysis provided further mechanistic insights into the physiological effects of dietary supplementation. Variations in enzymatic activity, glucose, lipid profiles and protein metabolism reflect the hens' metabolic responses to different dosages of purslane and vitamin E, offering potential biomarkers for assessing dietary efficacy and safety (Johnson, Smith, & Davis, 2021). High doses of purslane and vitamin E were associated with elevated liver enzymes, triglycerides and cholesterol, suggesting possible hepatotoxic effects and disruption of lipid metabolism. Conversely, moderate supplementation maintained metabolic parameters within physiological ranges, indicating potential benefits without compromising hen health (Islam et al., 2023).

Beyond immediate physiological outcomes, the dietary supplementation may influence egg quality and nutrient composition, with implications for market value and consumer acceptance. The functional foods such as purslane-enriched eggs could meet the growing demand for nutrient-dense poultry products; however, sensory evaluations and consumer studies are necessary to ensure palatability and commercial feasibility (Clark & Wilson, 2022).

The study also underscores the dynamic nature of poultry nutritional requirements which are influenced by genetic, environmental and production factors. The precision nutrition and omics-based approaches may provide promising avenues for tailoring diets to individual flocks, optimizing health, productivity and sustainability (Thomas & Garcia, 2023). Additionally, strategic dietary interventions can enhance human nutrition by improving the nutrient content of poultry products and reduce the environmental footprint of poultry farming through more efficient feed utilization and waste management (Smithson et al., 2021; Jones & Brown, 2020). Overall, the findings demonstrate a complex interplay between dietary supplementation, intestinal health, and systemic physiological responses. Evidence-based formulation of poultry diets with careful attention to dosage is essential for promoting animal welfare, optimizing growth and ensuring sustainable production (Khalid et al., 2024). Further research is warranted to refine dosage regimens and evaluate long-term effects on poultry performance and product quality.

Conclusion

The present study evaluated the effects of vitamin E and purslane supplementation on the intestinal health and overall physiology of golden hens, providing key insights into dietary strategies for improving poultry health and product quality. The findings demonstrate that these dietary interventions can positively influence multiple physiological parameters, including intestinal morphology, immune function and oxidative stability.The supplementation with vitamin E and purslane led to improvements in gut health, as evidenced by enhanced villus height, increased crypt depth, and a favorable shift in gut microbiota composition toward beneficial bacterial populations. These changes suggest enhanced nutrient absorption, improved intestinal function, and a strengthened immune response as reflected by elevated levels of immunoglobulins and cytokines. Furthermore, the

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results highlight the potential for these dietary strategies to enhance poultry productivity and disease resistance, supporting their application in sustainable poultry farming. Future investigations should explore the effects of these supplements on the nutritional quality of eggs and meat, assess their impact across different poultry species, and ensure that supplementation does not introduce harmful residues or pose risks to human health. Collectively, this study underscores the importance of optimizing dietary supplementation to improve both poultry welfare and the nutritional value of poultry-derived products.

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