Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com

Intestinal mucosal morphology and microbial flora population in *Arian* strain broilers fed with *Spirulina platensis* supplemented drinking water

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A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Ramezanpour Shahi, A., Mamashli, M., & Ghorbani, B. (2025). Intestinal mucosal morphology and microbial flora population in *Arian* strain broilers fed with *Spirulina platensis* supplemented drinking water. *Journal of Poultry Sciences and Avian Diseases, 3*(1), 54-62.
http://dx.doi.org/10.61838/kman.jpsad.3.1.5

A B S T R A C T

The aim of this study was to evaluate the impacts of different levels of *Spirulina platensis* supplementation on the mucosal morphology of the intestine of broiler chickens. Spirulina, a single-celled algae, has been recently categorized as a filamentous, gram-negative *Cyanobacterium*, which offers significant benefits in broiler nutrition by improving growth performance through its high protein content, enhancing immune function, boosting feed conversion efficiency, and supporting gut health. A total of 375 one-day-old male *Arian* broilers were included in a 6-week trial. The chicks were randomly assigned to five treatment groups, each with five replicates of 15 birds, received different concentrations of Spirulina in their drinking water: Group A (5 g/L), Group B (2.5 g/L), and Group C (1 g/L), while the control groups received either no additive or an antibiotic (Fosbac Biotic®). This study examined the effects of dietary *Spirulina platensis* supplementation on cecal, the height of intestinal villi, and the depth of crypts in Arian strain broiler chickens examined. The results indicated Spirulina increased villus height, reduced crypt depth, and improved the villus-to-crypt ratio in the duodenum jejunum and ileum, suggesting enhanced nutrient absorption. Moreover, Spirulina supplementation increased beneficial bacteria (lactobacilli) and reduced harmful bacteria (coliforms), improving overall gut health and reducing digestive problems. Further research is advised to explore the economic benefits of using Spirulina in the poultry industry.

Keywords: *Spirulina, Broiler chickens, Arian strain, Poultry, Microbial flora*

Article history:
Received 17 July 2024
Revised 27 September 2024
Accepted 04 October 2024
Published online 01 January 2025

1 Introduction

One of the primary concerns in poultry farming is using medications as feed additives. The application of medications was banned in Europe back in 2006 due to worries about the development of resistance to pathogens and their long-lasting presence in animal tissues (1). As a result, there is a quest for a feasible substitute for medications in chicken farming. Medicinal plants and essential oils derived from plants hold particular significance due to their ability to combat microbes and their positive impact on the digestive system of animals (2). The advantageous impacts of herbal supplements on livestock could be attributed to their ability to boost food intake, stimulate the immune system, combat microbes, and possess antiviral or anti-inflammatory properties(3). Food supplement sources should be high in protein content, contain well-balanced and specific amino acids, be easily digestible, and be safe for the bird's nutrition. It would be ideal if these items included extra innate characteristics such as simple digestibility, necessary nutrients, essential minerals, and tiny elements. Spirulina, a blue-green algae, is well-known for its remarkable nutritional profile, rich in proteins, vitamins, minerals, and antioxidants. Packed with proteins, vitamins, minerals, and crucial fatty acids, Spirulina has been shown in past research to improve immune response, antioxidant levels, and overall growth in birds (4). Thanks to its high protein content and positive effects on the breakdown and uptake of nutrients in young chickens raised for their meat, Spirulina is a helpful replacement for amino acids. In addition, its beneficial antioxidant and probiotic qualities assist in preventing a variety of gastrointestinal problems. Due to its elevated protein levels and favorable impacts on the digestion and absorption of food in young chickens bred for meat, Spirulina serves as a beneficial substitute for amino acids. Its favorable antioxidant and probiotic characteristics also aid in preventing various gastrointestinal issues (5). Studies have shown that Spirulina algae have alkaloids, flavonoids, sugars, tannins, phenolic substances, hormones, and foaming agents (6, 7). Researchers have found that young chickens given food with a Spirulina addition have higher body mass and better growth rates. (8). Apart from the conversation about the care of farm animals, another significant topic nowadays is the utilization of dietary additives to boost development and improve the nutrient quality in their food for human consumption. Using antibiotics as growth promoters in animal feed gained popularity in the 1950s to improve weight gain, boost food production, and support the overall health of poultry. However, because of issues such as the emergence of antibiotic resistance in pathogens, the buildup of antibiotics in consumable tissues, and their effect on human microbial communities, guidelines were implemented to limit their usage in poultry diets (9, 10). As this issue in animal farming persists, multiple areas have banned using antibiotics as enhancers of growth in animal nourishment. Instead, suggestions have been made for incorporating probiotics, natural acids, oligosaccharides, and additional dietary enhancements (11, 12). In addition to the mentioned factors, animals' health and immunity systems play a crucial role in their performance. A compromised or stressed immune system can lead to reduced daily progress, while a robust immune system can improve overall performance. Therefore, the use of immune system boosters could enhance overall efficiency. The aim of this study was to evaluate the effect of *Spirulina* microalgae as a feed ingredient source in the gut composition and microbial community of *Arian* strain broilers. Understanding that the gut flora plays a vital role in maintaining gut wellness and warding off illnesses is crucial. A minor feature of the digestive system plays a crucial role in nutrient processing and absorption, vital for maintaining overall health. Changes to these structures can significantly affect how nutrients are absorbed and can significantly impact the health of animals.

2 Materials and Methods

In this study, 375 one-day-old *Arian* broiler chickens with body weight (41.5 ± 0.5 g) were randomly assigned to six treatments (each group includes 15 chickens) with five replicates. The vaccination program was set up in the same way according to the *Arian* strain broiler manual guide, the information of which is recorded in Table 1. Based on the requirements of the *Arian* strain, the diet was formulated based on the broiler manual guide outlined in Table 2. As per this nutritional regimen, a starter diet pellets were administered from day 1 to 10, followed by a grower diet pellets from day 11 to 23, a first finisher diet pellets of 1 from day 24 to 35, and second finisher diet pellets was implemented from day 36 to 42.

The rearing system was located in a controlled research environment with regulated temperature and lighting. The broilers were provided with unlimited access to food and water throughout the experiment. During the first week, the temperature was maintained at 33 °C, gradually lowering to 22 °C in the fifth week. The relative humidity was maintained between 50 and 60 percent.

During the initial 14 days of the rearing period, a solution of commercial spirulina powder prepared was added to drinking water as follows: Group A: Spirulina 5 g/L, Group B: Spirulina at 2.5 g/L, Group C: Spirulina 1 g/L, Group N: Negative control (No additive), Group P: Positive control (Fosbac Biotic® 80 mg/kg) (Table 3). Spirulina powder prepared by Degzha company, Iran.

On day 14 of the rearing period, fecal samples from each pen were collected separately. One gram was blended with 9 mL of sterile peptone water and mixed for 1 min on a vortex. A 10-fold serial dilution (ranging from 10-1 to 10-8) was performed to count viable bacteria. The diluted samples were then plated on *Lactobacillus* MRS agar plates and McConkey agar plates to isolate *Lactobacillus* and *coliform* bacteria, respectively. Then the Lactobacillus agar plates were incubated for 48 hours at 37°C under anaerobic conditions, while the McConkey agar plates were incubated for 24 hours at 37°C under aerobic conditions. After the incubation periods, colonies of the respective bacteria were counted and expressed as the logarithm of colony-forming units per gram (log10 CFU/g).

At the end of the trial (day 45), five birds were selected from each treatment that closely matched the average weight of their respective groups. These birds were euthanized to prepare a histological sample from the small intestine, and 3 cm segments of the intestinal tissue from the ileum, jejunum, and duodenum that were collected to prepare histological samples. Then, the collected samples were sent to the laboratory to be preserved in 10% formalin to prepare pathology slides. Villus height and crypt depth were measured using light microscopy and imaged with a microscope connected to a computer. The villus-to-crypt ratio was calculated to assess the absorptive capacity of the intestine.

The statistical analysis was conducted using the One-Way ANOVA method with SPSS software (Version 27; SPSS Inc., Chicago, USA) at a 5% confidence level.

Table 1. Broiler vaccination program, Arian strain

Vaccine	Age	Dosage	Route of Administration
B1-H120	1	1	Eye Drop
IB 4/91	1	1	Eye Drop
AI-ND	7	1	Subcutaneous
ND Clone 30	7	1	Eye Drop
ND Clone-H120	12	1	Drinking Water
IBD D78	14	1	Drinking Water
ND Clone 30	20	1	Drinking Water
IBD D78	24	1	Drinking Water

Table 2. Ingredient composition of experimental diets

Finisher 2 (kg)	Finisher 1(kg)	Grower(kg)	Starter (kg)	Items
621.8	589.72	539.12	473.12	Corn
270	300	350	380	Soybean meal
20	18	16	14	Soybean oil
50	50	50	50	Grain
9	10	11.5	18	D- Calcium phosphate
2	2	2	2	Salt
2.5	5/2	2.5	2.5	Sodium bicarbonate
9	9	9	9	Calcium carbonate
-	-	-	30	Corn Gluten
2	4/2	2.9	3.2	Methionine
2	1/2	2.1	2.8	Lysine
0.6	8/0	0.9	1	Threonine
-	5/0	0.6	0.7	Colin
-	08/0	0.08	0.08	Phytase
-	2/0	0.3	1	Valine
1	1	1	3.03	Toxin Binder
5	5	5	5	Plette Binder

Table 3. Nutrient composition of *Spirulina platensis* (13)

%	Items
60-55	Protein
0.01	Fiber
15- 18	Carbohydrate
7.5 - 12	Lipid
7-9	Dry matter
4- 6	Moisture
0.2 – 0.29	Carotinoid
0.7 – 0.8	Clorophil α

3 Result

Adding spirulina powder into the water positively influenced the villi's height and the ratio of villus height to crypt depth in the duodenum and jejunum. Image No. 1-4. Using Spirulina in their diet resulted in a significant increase in villus height and the villus height to crypt depth ratio compared to the group that had antibiotics in their drinking water (group P) and the group without Spirulina (group N). Birds that received Spirulina also had higher villi in the jejunum than those that received antibiotics, and the ratio of crypt depth to villus height was improved in the jejunum. In the ileum, *Spirulina* increased villus height and improved the villus height to crypt depth ratio compared to the groups that had received antibiotics and the negative control group. There was no significant difference between treatment groups that used different percentages of Spirulina in drinking water (Table 4).

Adding different levels of Spirulina (5, 2.5, or 1 g) to the drinking water of *Arian* chickens increased beneficial intestinal lactobacilli in broiler chickens. Additionally, compared to the negative control group and the positive control group, the total intestinal flora population in chickens fed with *Spirulina* decreased. Moreover, the use of *Spirulina had* an adverse effect on the population of *E.coli* in the small intestine (Table 5).

Table 4. The effects of Spirulina on the morphology of the small intestine

Treatments	Section								
	Duodenum			Ileum			Jejunum		
	H	CD	CD/H	H	CD	CD/H	H	CD	CD/H
A	2091.68[a]	1921.479[c]	1.088	1134.41[bc]	789.759[c]	1.453	1423.024[a]	1037.653[c]	1.371
B	2001.59[a]	1899.65[ac]	1.053	1168.19[a]	801.33[ac]	1.457	1408.130[a]	1048.11[ac]	1.343
C	1981.67[b]	1901.78[bc]	1.042	1056.89[b]	798.65[b]	1.323	1398.78[b]	1076.22[b]	1.299
P	1842.35[b]	1627.63[ab]	1.131	989.31[b]	683.89[ab]	1.446	1342.12[c]	989.45[ab]	1.356
N	1761.36[b]	1584.41	1.111	975.79[b]	680.24	1.434	1311.62[c]	976.51	1.341
SEM	16.43	2.18	0.18	22.41	1.38	0.16	14.53	1.98	0.17
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.013	<0.001

⁎ Values within columns with no common superscripts differ significantly ($p < 0.05$)

Table 5. The effects of *Spirulina* on bacterial population in small intestine

Treatments	Log CFU/g		
	Coliforms	*Lactobacillus*	*E-coli*
A	6.02[c]	8.04[a]	6.14[b]
B	7.09[b]	7.23[b]	6.23[ab]
C	7.11[b]	7.05[b]	6.17[ab]
N	7.43[b]	6.52[b]	6.67[a]
P	7.96[a]	5.98[c]	6.95
SEM	0.12	0.13	0.17
p-value	<0.001	<0.001	<0.001

⁎ Values within columns with no common superscripts differ significantly ($p < 0.05$)

Figure 1. Histology section from the jejunum in Spirulina Group chickens (Groups A, B, and C)

Figure 2. Histology section from jejunum in chickens without *Spirulina* (Groups P and N)

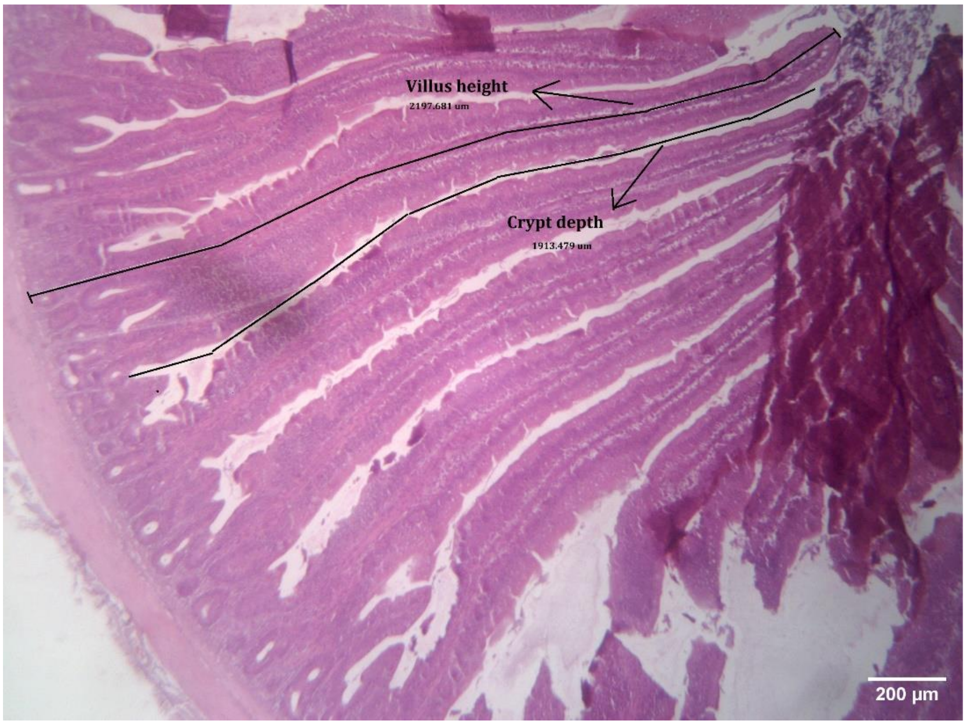

Figure 3. Histology section from duodenum in Spirulina Group chickens (Groups A, B, and C)

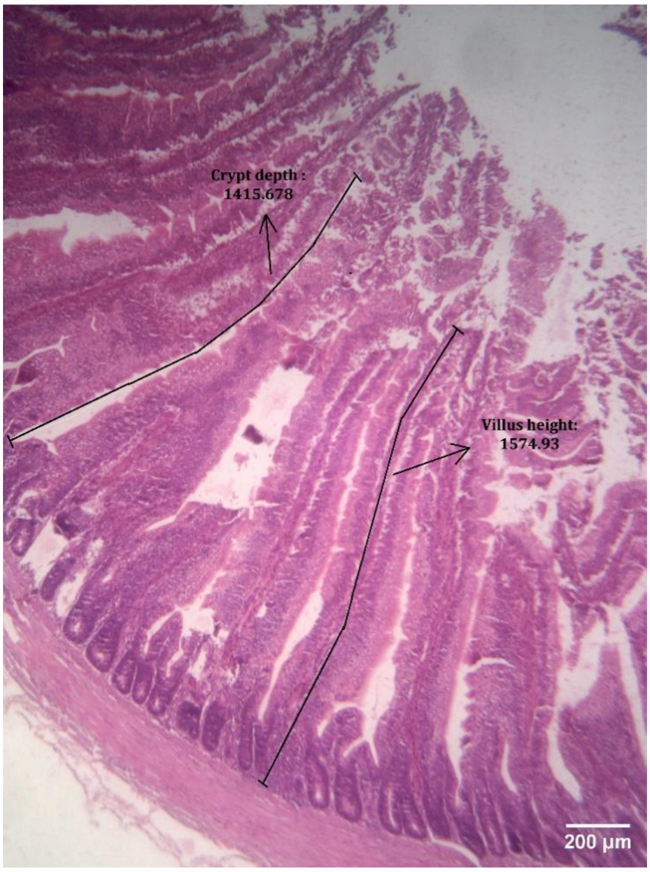

Figure 4. Histology section from duodenum in chickens without *Spirulina* (Groups P and N)

4 Discussion

Poultry farming faces the significant challenge of finding effective food supplements and alternatives to promote growth, enhance immune system function, and boost the nutritional value of poultry products for human consumption without resorting to antibiotics. De ovliuria et.al. have proved that spirulina algae are a high-quality feed additive suitable for poultry nutrition (14). In this research, one novel strategy was administering Spirulina in the drinking water rather than in the feed. This approach not only simplified the administration process but also resulted in increased economic benefits by lowering feed costs. Also, this study was examined on an *Arian* strain broiler for the first time. Spirulina has been found to enhance the tissue structure of the intestine by increasing the thickness of the intestinal wall and the number of goblet cells while also reducing inflammation in the intestinal tissues. These beneficial effects can result in improved nutrient absorption and enhanced growth efficiency in chickens. Moreover, Spirulina protects against intestinal damage induced by stress and diseases (15). This study observed similar structural improvements, specifically the increase in villus height and crypt depth, that contribute to more efficient nutrient absorption. Spirulina has been shown to enhance the absorption of nutrients in the diet by elevating the activity of digestive enzymes and improving absorption efficiency in the intestinal wall (16). This research showed that Spirulina can protect the intestinal structure by maintaining villus integrity. This reduces intestinal stress in broilers. The consumption of Spirulina has been shown to enhance the digestive function in chickens, leading to increased nutrient absorption. This improvement is attributed to heightened digestive enzyme activity and enhanced absorption efficiency within the intestinal wall. Incorporating Spirulina into their diet can effectively boost feed efficiency and promote better overall health in broiler chickens (17, 18). Through enhancing protein digestibility, Spirulina can contribute to the improved metabolism of proteins in chickens. These positive changes may stem from heightened proteolytic enzyme activity and enhanced absorption of amino acids in the intestines. Integrating Spirulina into their diet can potentially boost growth efficiency and enhance the body composition of broiler chickens (19). The utilization of Spirulina has been shown to have positive effects on the reduction of intestinal diseases in broilers, while also contributing to their overall health (20). Similarly, this study observed an increase in the population of beneficial *lactobacilli* and a reduction in harmful coliforms in *Arian* strain broilers, leading to enhanced gut health and immunity. Broilers containing Spirulina have shown better growth performance, including increased body weight and improved Feed Conversion Ratio. These improvements could be due to increased nutrient absorption and improved chickens' overall digestive health (21). The utilization of spirulina supplements has been shown to have the potential to enhance general health and decrease the reliance on antibiotics in broiler farming (11). This aligns with the findings of this experiment, which state that Spirulina enhanced intestinal structure and shifted the gut microbiota in favor of beneficial bacteria, resulting in overall digestive and immune health in *Arian* strain broilers. Various theories have been suggested to explain the positive effects of Spirulina on histology and intestinal microbial flora. One of these mechanisms is Spirulina's antioxidant activity, which helps reduce oxidative stress in intestinal tissues, preventing cell damage and promoting overall body health. Spirulina is known to enhance the immune system and boost the production of natural antimicrobial substances in chickens (22, 23). Ciferri et al. demonstrated that Spirulina could increase the proliferation of beneficial bacteria, such as *lactobacilli* and *bifidobacteria*, while reducing harmful bacteria, such as *coliforms*. These shifts in intestinal microbial flora can contribute to better digestive health and increase resistance to intestinal diseases, which is completely consistent with the findings of this study, in which harmful bacteria generally decreased and beneficial lactobacilli increased. Research shows that Spirulina can promote a more balanced intestinal microbial environment and significantly enhance the body's immune system (24, 25). The study's findings demonstrate the positive effects of Spirulina as a food supplement in the drinking water of *Arian* strain broilers. This research uniquely focused on using Spirulina only in the first two weeks of broiler growth, which is vital in a chicken's development. Limiting Spirulina to the first two weeks instead of the entire growth period achieves significant cost savings while effectively improving intestinal structure. The results indicate improvements in intestinal structure, such as increasing the height of the villi, reducing the depth of the crypts, improving the height of the villi to the depth of the crypt ratio in the duodenum, jejunum, and ilium, and the increase in beneficial bacteria (*Lactobacillus*) and the reduction in harmful cells (coliforms), leading to enhanced overall health and performance of the chickens. Spirulina at a rate of 2.5 g/liter yielded the best economic results, suggesting its potential as a valuable supplement in broiler chickens. We hypothesized that spirulina supplementation would maintain the beneficial microbial population and subsequently explain this study's improved digestibility and growth performance. Furthermore, the study recommends additional research to explore the precise mechanisms by which Spirulina affects intestinal structure and function and the long-term effects of its inclusion in broiler diets. Investigating the economic benefits of using Spirulina in poultry production is also highlighted as essential, along with further exploration of its potential impact on broilers' immune systems, meat quality, and overall performance indicators.

5 Conclusion

Including Spirulina in *the Arian* strain in broiler diets can lead to enhancements in intestinal tissue structure and favorable alterations in the population of intestinal microbial flora. These outcomes may contribute to the overall health improvement and enhanced growth efficiency of broiler chickens. Additional research is required to understand the specific mechanisms behind these effects and establish the ideal spirulina dosage in dietary formulations.

Acknowledgements

We thank ETKA Organization and Mazrae Nemuneh Integration for supporting the current study.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

Authors equally contributed to this study.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

The Animal Welfare Committee of ETKA Organization approved all experimental procedures.

Funding

The Mazrae Nemuneh Integration financially supported this study.

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