Adding seaweed Spirulina (Arthrospira platensis) to feed and its effect on histological and microbial characteristics of Ross 308 broiler chickens

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Abstract.
This study aimed to determine the effect of adding Spirulina (Arthrospira platensis). algae powder to feed on some histological and microbial characteristics of the intestines of Ross 308 broiler chickens. 225 birds were used in this study. The birds were randomly assigned to five experimental groups, with three replicates per group, each containing 15 birds. The first experimental group (T1) received a standard feed without any additives, while the second (T2), third (T3), fourth (T4), and fifth (T5) received a standard feed supplemented with 7.5, 15, 22.5, and 30 grams of Spirulina (Arthrospira platensis). kilogram of feed, respectively. After 35 days, the total number of birds used for sampling, 45, was divided among the five experimental treatments, with 9 birds per treatment per replicate. Birds were slaughtered, and tissue sections were taken to study tissue characteristics, including villus length and width and crypt depth. A microbial section was also taken from the slaughtered birds to estimate total bacterial counts, coliform bacteria, and lactic acid bacteria The results showed a significant effect ($P \leq 0.05$) of the experimental treatments on villus length and width compared to treatment 1 (T1). Treatment 5 (T5) outperformed the other treatments in terms of villus length and width, recording the highest values of 840.44 and 178.11 μm, respectively A significant effect ($P \leq 0.05$) was also found between total bacterial counts, coliform bacteria, and lactic acid bacteria on the overall bacterial count quality Treatment T3 showed a significant advantage ($P \leq 0.05$) over the other experimental treatments in terms of total bacterial count, recording the highest value of 66.25 colony-forming units (CFU/g). Regarding the number of harmless lactic acid bacteria, treatment T5 showed a significant advantage ($P \leq 0.05$) over the other treatments, recording a value of 58.00 CFU/g. As for the number of coliform bacteria, treatment T1 recorded the highest value of 39.43 CFU/g compared to the other experimental treatments.
Keywords. broilers, Seaweed powder (spirulina), Histological, microbial.

1. Introduction:

The increased demand for animal protein is due to recent population growth. Consequently, there is a growing need to improve production rates, particularly for poultry meat, given its rapid life cycle and affordable price [1]. Adding seaweed as a feed supplement to poultry feed provides a source of amino acids, especially essential ones, necessary for growth and development. Seaweed is a sustainable natural resource, containing essential nutrients for poultry nutrition, such as vitamins, particularly vitamin B12 and vitamin A. Seaweed also acts as an antioxidant, enhances immunity and growth in broiler chickens, improves gut microbiome and absorption, and increases sexual maturity [2, 3]. Seaweed is classified into three groups, comprising approximately 7,000 red species, 2,000 brown species, and 1,000 green species [4]. Several studies have shown that adding seaweed to poultry feed leads to increased body weight, improved feed conversion ratio, and a desirable yellow coloration in the legs and skin, which is desirable to consumers [5]. It reduces heat stress and blood cholesterol, reduces the level of low-density lipoproteins (LDL), increases the level of high-density lipoproteins (HDL), and increases the thyroid hormones T3 and T4, thus improving the physiological condition of the

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bird and improving the internal environment in the intestines by increasing the number of beneficial bacteria and reducing the number of harmful bacteria [6]. Because of these active substances found in seaweed, researchers have become increasingly interested in them in the fields of cosmetics, fertilizer manufacturing, medicine, and many other fields [7]. These active ingredients have benefits in improving the bird's immunity and act as anti-inflammatory, anti-cancer and growth stimulants, it also works as an antioxidant, antibacterial and antiviral [8,9]. Inhibiting oxidative stress, improving the internal intestinal environment and enhancing the body's immunity [10,11]. The effect of seaweed on body weight gain, feed consumption, improving liver and kidney functions, and enhancing immunity [12]. It also improves the digestion and absorption of nutrients in the intestines. This is due to the presence of non-starch sugars such as cellulose and hemicellulose, which interfere with the digestion and absorption of nutrients [13]. There is a positive correlation between the consumption of feedstuffs containing seaweed and the growth rate of birds, the increase in the size of the villi, and the increase in the width and height of the villi in the intestine, thus improving the process of digestion of nutrients and absorption due to the increase in the surface area of the absorption surfaces [14]. The active compounds in seaweed prevent the growth of pathogenic or harmful bacteria such as *E. coli* and *Salmonella enterica* and enhance the growth of beneficial bacteria in the intestines (lactic acid bacteria), thus improving the work and functions of the intestines and ultimately improving the growth performance of poultry [15]. Adding seaweed powder to poultry feed resulted in an increase in the length and width of villi and the depth of crypts. The birds also enjoyed good intestinal health compared to birds in other treatments this leads to an increase in the surface area of the absorption surfaces of nutrients within the digestive system, and thus a better increase in the rate of food utilization, as these cells secrete important substances to enhance the internal environment of the intestine [16,17].

2. Materials and Methods:

Source of seaweed powder (spirulina):

The seaweed (spirulina) was obtained from one of the herbalists' shops in the local markets, and it was imported from the American company Aco in the form of a powder.

The experiment was conducted in the poultry field of the Department of Animal Production, College of Agriculture, University of Diyala, from 9/24 to 10/28/2024 to study the effect of adding seaweed powder (Spirulina) to the diet on the histological and microbial performance of broiler chickens (Ross 308). The birds were 225 unsexed chicks at one-day old average weight 37.5g. They were randomly divided into five treatments with three replicates and 15 birds/replicate. They treatment was:

- (T1): Control: Diet without any additives.
- (T2): Standard Diet with Spirulina (7.5) g. / kg of diet.
- (T3): Standard Diet with Spirulina (15) g./kg diet.
- (T4): Standard Diet with Spirulina (22.5) g. /kg diet.
- (T5): Standard Diet with Spirulina (30) g. /kg diet.

The chicks were raised in a semi-closed hall, in floor cages with an area of 2 x 1.5 m for each cage (15 birds/cage). The floor of the cages designated for the chicks was covered with sawdust approximately 5 cm thick and litter paper until the end of the first week. In the first week of rearing, feeders in the form of plastic plates and inverted plastic troughs were used. At the end of the first week, the plastic feeding dish was replaced with a circular, hanging dish with a diameter of 45 cm, as well as automatic plastic dishes trough instead of the inverted trough until the end of the marketing age (five weeks). The light program of 23 hours

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of light and 1 hour of darkness was used during the first three days. After that, a program of 20 hours of light and 4 hours of darkness was applied until the marketing age of five weeks, as indicated by Company Directory [18]. The chicks were received in the field at a temperature of 33-34°C on the first day, and then temperature was reduced Every day 0.5°C to reach a temperature of 21°C in the third week, and it was fixed until the marketing age according to the Aviagen breeding guide [18]. The birds were fed a starter diet from 1-10 days, a grower diet from 11-24 days, and a finisher diet from 25-35 days old. The starter diet contained 23% crude protein and 2975 kcal/kg energy, grower diet contained 21.5% crude protein and 3050 kcal/kg energy, and finisher diet contained 19.5% crude protein and 3100 kcal/kg energy. The Seaweed (spirulina) was obtained by importing it from the American company Eco.

Table 1. Chemical analysis, amino acids, vitamins and minerals in marine algae (spirulina).

Ingredients	Concentration (g/100g)	Alanine	39.36
		Aspartate	13.63
Crude Fiber	3.22	Vitamins	Concentration (mg/1g)
Moisture	12.2		
Protein	53.78	Vitamin B2	40.45
Fat	5.92	Folic acid	74.62
Ash	9.67	Vitamin B12	282.06
Amino Acids	Concentration (mg/1g)	Vitamin C	181.42
		Vitamin A	N. D
Arginine	79.07	Vitamin D3	N. D
Histidine	11.99	Vitamin E	96.06
Isoleucine	10.73	Metals	Concentration (g/100g)
Leucine	45.81		
Lysine	32.00	Lead (Pb)	3.58
Methionine	15.28	Phosphorus(P)	0.75
Phenylalanine	37.93	Potassium (K)	1.44
Tryptophan	5.97	Sodium(Na)	1.53
Threonine	N. D	Copper (Cu)	9.83
Valine	N. D	Nickel (Ni)	Nil
Glycine	16.84	Iron(Fe)	0.049
Glutamate	62.66	Mercury (Hg)	Nil
Serine	29.22	Cadmium (Cd)	Nil

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ND: Not determined [19]

The Data were statistically analyzed using a completely randomized design (CRD), The significant differences of the means were determined by Duncan's multiple range test [20] at the 0.05 level [21]. The ready-made SPSS statistical program was also used to analyze the experimental data [22].

3. Results and Discussion:

3.1 Histological properties of the intestine:

It is clear from Table (2) that there are significant differences (P≤0.05) between the different treatments in the studied histological traits, which are the trait of villi length and width in broiler chickens at the 35 day old, It was noted through the results of the statistical analysis that the T5 was superior in the length and width of the villi, as values of 840.44 and 178.11 µm were recorded for the two properties respectively, compare with T1, T2 and T3, While there was no significant difference with the T4, the T1 recorded the lowest values for the two studied traits, which were 617.40 and 137.20 µm for the traits of villus length and width, respectively, and no differences were recorded between the other treatments. On the other hand, the results of the statistical analysis presented in the same table did not record any significant differences P ≤ 0.05 in the trait of crypt depth at the age of 35 days. The superiority in the length of the broiler intestines of the different experimental treatments compared to the T1 may be due to the effect of adding Spirulina in improving the body weight gain, increasing feed intake, improving liver and kidney functions, and enhancing immunity [12]. It also improves the digestion and absorption of nutrients in the intestines, due to the presence of non-starch sugars such as Cellulase and Hemicellulase, which interfere with the digestion and absorption of nutrients [13]. Spirulina also acts as antioxidants and immune boosters, reducing the formation of free radicals that damage the body's cells, including villus cells, and improving growth and sexual maturity in broiler chickens [2,3]. The results were consistent with what [16] found, that adding algae powder (Spirulina) to poultry feed led to an increase in the length and width of the villi and the depth of the crypts, and the birds also enjoyed good intestinal health. Improvement leads to an increase in the surface area of the nutrient absorption surfaces and thus better utilization of food, as these cells secrete important substances to enhance the internal environment of the intestine [17].

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Table 2. Effect of adding (Spirulina) powder on the length, width, and depth of villi (micrometers) of the intestines of 35-day-old broiler chickens (Mean ± S.E).

Treat. *	Villus length µm	Villus width µm	Crypts Depth µm
T1	617.40±33.46 b	137.20±8.46 b	79.40±3.35
T2	657.65±33.64 b	144.29±7.96 b	86.18±1.46
T3	623.30±69.32 b	133.35±10.57 B	84.15±2.67
T4	702.40±60.45 ab	164.13±6.12 A	86.80±2.54
T5	840.44±53.34 a	178.11±7.65 A	86.41±1.19
P-Value**	0.022	0.001	0.305

*T1, T2, T3, T4 and T5 contained (0, 7.5, 15, 22.5 and 30) g Spirulina /kg diet supplemented.
** Means within the same column with different superscripts differ significantly ($p < 0.05$).

3.2. Microbial Properties of the small intestine:

Table (3) showed s significant differences ($P \leq 0.05$) between the all treatments in the Total of bacterial count, Lactic acid bacteria, and Coliform bacteria, at the age of 35 days. It was noted through the results of the statistical analysis that T3 and T4 were superior in the total bacterial count, were recorded 66.25 and 65.75 CFU/g, respectively, compared with T1, T2 and T5, while the T1 recorded the lowest value 53.25 CFU/g. As well as for the number of lactic acid bacteria, the results showed a significant superiority ($P \leq 0.05$) for T5 for this trait, as values of 58.00 CFU/g, were recorded in T1 and T3, while they did not differ significantly with T2 while T4 and T3 treatments recorded the lowest values for the number of lactic acid bacteria, which were 43.75 CFU/g, and As for the number of Coliform bacteria, it was noted through the results that T4 excelled in this trait, as it recorded the lowest values (30.06 CFU/g) compared with to other treatments, while the control treatment recorded the highest values for the number of Coliform bacteria, (39.43 CFU/g) and did not differ significantly with T3.

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Table 3. Effect of adding seaweed powder to broiler feed (Rose 308) on the bacterial counts in the intestinal flora (CFU/g) of broiler chickens at 35 days of age (mean ± S.E).

Treat. *	Total bacterial count	Lactic acid bacteria count	Coliform bacteria count
T1	53.25±1.97 b	47.75±0.81 b	39.43±0.81 a
T2	57.75±2.88 b	52.00±1.12 Ab	35.37±1.12 b
T3	66.25±3.15 a	43.75±2.39 b	37.81±2.39 ab
T4	63.75±2.83 a	56.75±2.85 a	30.06±2.85 c
T5	55.25±2.05 b	58.00±1.03 a	36.38±1.03 b
P-Value**	0.001	0.009	0.002

*T1, T2, T3, T4 and T5 contained (0, 7.5, 15, 22.5 and 30) g Spirulina /kg diet supplemented.
** Means within the same column with different superscripts differ significantly ($p < 0.05$).

The significant improvement in the experimental results in microbial characteristics, namely the increase in the number of beneficial bacteria and the decrease in the number of harmful ones, may be due to the fact that seaweed plays an important role through its effective compounds in reducing heat stress and the level of cholesterol in the blood, reducing the level of LDL, increasing the level of HDL, and increasing the thyroid hormones T3 and T4 this leads to improving the physiological condition of the bird and improving the internal environment of the bird's intestines by increasing the number of beneficial bacteria and reducing the number of harmful bacteria as a result of exclusionary competition [6], because of these active substances found in seaweed, researchers have become increasingly interested in introducing them into the fields of cosmetics, plant fertilizer manufacturing, alternative medicine, and other vital fields [7] these active ingredients have been shown to have significant benefits in improving and enhancing the general immunity of birds and acting as anti-inflammatory, anti-cancer, stimulant and growth promoter [8]. Their active compounds also act as antioxidants and antimicrobials against bacteria and viruses [9], inhibiting oxidative stress, improving the intestinal environment and enhancing immunity [10,11].

Conclusions.

The use of seaweed powder (Spirulina) in broiler diet increases the number of beneficial bacteria and inhibits the number of harmful bacteria in the intestinal tract.

The effectiveness of (Spirulina) added to broiler feed as an enhancer of the height and width of villi and the depth of crypts has also been demonstrated.

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