THE IRAQI JOURNAL OF
VETERINARY MEDICINE
eISSN:2410-7409
pISSN: 1609-5693
OPEN ACCESS

Enhancing the Productive Performance of Broiler Chickens by Adding *Spirulina platensis* Compared with Probiotic, Prebiotics, and Oxytetracycline

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Received: 10 November 2020
Accepted: 14 January 2021
Published: 28 June 2021

DOI:
https://doi.org/10.30539/ijvm.v45i1.1037

Cite:
Atiyah WR, Hamood MF. Enhancing the productive performance of broiler chickens by adding *Spirulina platensis* compared with probiotic, prebiotics and oxytetracycline. Iraqi J. Vet. Med. 28 June. 2021; 45(1): 31-36.

A B S T R A C T

This study was conducted in order to find out the effect of *Spirulina platensis* (*S. platensis*) algae mixed with drinking water (2%) on the production performance of broiler chickens infected with or without *Enterococcus faecalis* (*E. faecalis*) and compare it with the effects of probiotics (containing *Lactobacillus acidophilus*, *L. casi*, *L. r*euteri, and *Bacillus subtillis*), prebiotics (containing antioxidants and a group of vitamins) as well as oxytetracycline. The experiment included 350 one day old (Ross-308) broiler chicks, randomly divided into 7 groups by 50 chicks per group for 35 days. The groups were divided as follows: T_1: infected experimentally with *E. faecalis* only, T_2: treated with *S. platensis,* T_3: infected experimentally with *E. faecalis* and treated with 2% *S. platensis,* T_4: infected experimentally with *E. faecalis* and treated with probiotic, T_5: infected experimentally with *E. faecalis* and treated with prebiotic, T6: infected experimentally with *E. faecalis* and treated with oxytetracycline, and T_7: control group without any addition. Weekly live body weight and weekly body weight gain were measured as well as weekly feed intake and feed conversion rate for broiler chickens were estimated. The current study proved that *S. platensis* algae added to drinking water (2%) in T_2 and T_3 groups had caused a significant increase ($P<0.05$) in the weekly live body weight and weekly body weight gain, *Spirulina* also caused a significant decrease ($P<0.05$) in weekly feed intake and feed conversion rate for broiler chickens. Therefore, *S. platensis* could be a suitable alternative to some feed additives such as probiotics, prebiotics, and antibiotics in addition to the ability of *Spirulina* to reduce the symptoms of *E. faecalis*.

Keywords: *Spirulina platensis,* productive performance, broiler, probiotics, prebiotics

INTRODUCTION

Enterococcus faecalis (*E. faecalis*) is Gram-positive bacteria and facultative anaerobic constitute the natural microflora of intestinal tract in mammals and birds (1). *E. faecalis* is an opportunistic pathogenic bacterium that causes many infections in poultry including septicemia, endocarditis, central nervous system infections, salpingitis and amyloidosis (2). Antibiotics are used as growth promoters for poultry at low doses than therapeutic doses and the mechanics of antibiotic action is by interaction with intestinal bacterial mass (3-5). As a result of excessive use of antibiotics, they caused the appearance of new bacterial strains resistant to antibiotics (6). Significantly, *E. faecalis* antibiotic-resistant bacteria will develop rapidly (7-9). Resistance genes may be transmitted from animals to

humans through the food chain (10). *E. faecalis* bacteria may cause important infection and diseases to humans, such as endocarditis, bacteremia, inflammation of the urinary tract and central nervous system infections (11, 12).

When the use of antibiotics was banned in many countries of the world, it became necessary to search for feed additives of natural origin for poultry that would be safer and more acceptable for consumers (13). Phytogenic feed additives have been widely employed in the poultry industry to improve the health status and increase growth and productive performance (14).

Spirulina platensis (*S. platensis*) is a cyanobacteria (blue-green algae), which is considered as a rich protein food in addition to many vitamins and minerals (15). *S. platensis* has antioxidant and anti-bacterial properties as well as enhancing the immune system (16). *S. platensis* algae is considered as one of the most promising diets in the development of the poultry industry, and future research work must be done to exploit it for the purpose of increasing production at the lowest economic cost (17).

The experiment aims to determine the effectiveness of nutritional *Spirulina* algae as a natural component on the production performance of broiler chickens.

MATERIALS AND METHODS

Experimental Design

The experimental design of the study was based on the approval of the Public Health Department at the College of Veterinary Medicine, University of Baghdad, in its session held on February 25, 2019. All procedures carried out in this study were reviewed and accepted in compliance with the ethical principles of animal welfare by the Scientific Committee at the College of Veterinary Medicine, University of Bagdad.

This study was conducted in the poultry fields of the College of Veterinary Medicine, University of Baghdad for the period from November 9, 2019, to December 13, 2019. The experiment included a total of 350 one-day-old straight-Run broiler chicks (Ross-308), which were randomly divided into 7 groups with 50 chicks per group placed in 2×2 m^2 pens for 5 weeks.

The challenge test was pre-performed on 5 groups, each group consisted of 20 chicks (they were challenged with different concentrations of bacteria to choose the concentration that killed half of the experimental animals) to determine the LD_{50} of bacteria, which was 1×10^8 CFU/mL. Chicks of groups T_3, T_4, T_5 and T_6 were infected on the third day of their life, then the clinical symptoms were observed in the chicks in the second week of life (18).

T_1 = *E. faecalis.* only, T_2= added *S. platensis*, T_3 = *E. faecalis.* + *S. platensis* (2%), T_4 = *E. faecalis.* + probiotic (DPP company, USA, contains minimum of 1 x 10^8 CFU/g total *Lactobacillus acidophilus, L. casi, L. reuteri, and Bacillus subtillis* 3.3 g/20 liters of drinking water), T_5= *E. faecalis.* + prebiotic (produced by Biolink Company, UK, contains extract of beta-glucans as antioxidants and a group of vitamins 7.5 mL in 20 liters of clean drinking water), T_6= *E. faecalis.* + oxytetracycline 2500 mg/L of drinking water, T_7: control group without any addition.

Table 1. The nutritional value of *Spirulina* algae used per 100 g

Nutritional content	Per 100 g
Energy	332 kcal
Fat	0.8 g
Carbohydrate	12.8 g
Protein	65.3 g
Fiber	6.4 g
Salt	3 g
Vitamin A	1200 µg
Riboflavin (B2)	0.3 mg
Vitamin B6	0.7 mg
Magnesium	2.59 mg
Iron	53.2 mg
Chromium	22 µg

Mean Body Weight (g)

The body weights were calculated weekly by weighing chicks individually at day one old and at the end of each week by sensitive balance. Mean body weight was calculated from the weight of all chicks divided on the number of chicks (19).

Weekly Mean Weight Gain (g)

The mean body weight gain (WG) for each group was calculated weekly by recording the weight gain at the beginning of the week and at the end depending on the following equation (20):

$$\text{Weekly WG} = \text{BW at end of week} - \text{BW at begining of week}$$

Feed Intake

Feed intake (FI) was calculated weekly depending on weighing the remained feed at number of the dead chicks and the number of their feeding days. Feed intake of chicks was calculated according to the equation which was mentioned by (21) as follows:

$$\text{Weekly FI (g/chick)} = \frac{W}{L+D}$$

Where, W = quantity of FI through the week (g), L= number of live chicks fed through the week., D = numbers of dead chicks × number of their feeding days.

Feed Conversion Ratio

Feed conversion ratio (FCR)was measured weekly for each group up to the end of experiment. Measurement was done according to the equation below (19):

$$\text{FCR (g:g)} = \frac{\text{FI (g)}}{\text{BW (g)}}$$

Statistical Analysis

Statistical Package of Social Sciences (SPSS) version 26 (Inc., Chicago, IL, USA) computer software was used for analysis of the results. The data were analyzed as one-way ANOVA, and significant differences between groups were assessed using Fisher's Least Significant Difference (LSD). Values with P≤0.05 were considered to indicate statistically significance (22). All results are expressed as the mean±SEM.

RESULTS AND DISCUSSION

The nutritional analysis of *Spirulina* algae for every 100 g is shown in Table 1.

The results showed significant differences in the body weight of chicks in the first week of the experiment, where group T2 recorded a significant increase in body weight over the control group (P<0.05), while the other groups recorded a significant decrease compared with the control group (P<0.05). Group T_2 in all five weeks of the experiment recorded a significant increase over the control group (P<0.05). The other groups, on the contrary, recorded a decrease from the control group (P<0.05), this is also clearly shown in Table 2.

The results showed significant differences (P<0.05) between the treatment groups and the control group in weekly weight gain, where the weight gain for groups T_2 and T_7 was significantly more than the rest of the groups. The weight gain of the experimentally affected group T_3 increased significantly (P<0.05) more than the affected groups T_4, T_5 and T_6 as shown in Table 3.

Table 2. Effect of different levels of *Spirulina*, probiotic, prebiotic, and antibiotic with or without *E. faecalis* infection on weekly live body weight (g) of broilers[1]

	Period				
Treatment	**Week1**	**Week2**	**Week3**	**Week4**	**Week5**
T1	119±1.50 ^{De}	237±3.78 ^{Gd}	407±1.96 ^{Gc}	636±2.11 ^{Gb}	992±4.25 ^{Ga}
T2	147±2.25 ^{Ae}	413±4.56 ^{Ad}	886±2.73 ^{Ac}	1455±1.66 ^{Ab}	2115±7.54 ^{Aa}
T3	136±1.72 ^{BCe}	321±3.05 ^{Cd}	619±2.81 ^{Cc}	1006±2.55 ^{Cb}	1537±3.59 ^{Da}
T4	139±1.62 ^{ABe}	300±2.89 ^{Dd}	573±2.18 ^{Dc}	924±3.60 ^{Db}	1408±5.97 ^{Ca}
T5	127±2.70 ^{CDe}	271±1.96 ^{Ed}	508±2.54 ^{Ec}	782±3.19 ^{Eb}	1275±4.64 ^{Ea}
T6	126±2.41 ^{De}	250±1.79 ^{Fd}	493±3.80 ^{Fc}	748±1.52 ^{Fb}	1226±7.80 ^{Fa}
T7	143±1.15 ^{Abe}	396±2.00 ^{Bd}	825±3.15 ^{Bc}	1321±2.73 ^{Bb}	1880±6.38 ^{Ba}
$LSD_{0.5}$			9.43		

[1]Mean±SEM. T1 = *E. faecalis* only. T2 =: added *S. platensis* 2%, T3 = infected with *E. faecalis*. + *S. platensis* 2%. T4 = infected with *E. faecalis* + probiotic. T5 *E. faecalis* + prebiotic. T6 = *E. faecalis* + oxytetracycline. T7= Control. Different uppercase letters (A-G) refer to vertical statistical reading (among groups), lowercase letters (a-e) refer to horizontal statistical reading (among times) at P<0.05

Table 3. Effect of different levels of *Spirulina*, probiotic, prebiotic, and antibiotic with or without *E. faecalis* infection on weekly live body weight gain (g) of broilers[1]

	Period				
Treatment	**Week1**	**Week2**	**Week3**	**Week4**	**Week5**
T1	81±1.25 ^{Ee}	118±2.78 ^{Gd}	170±1.66 ^{Gc}	228±1.34 ^{Gb}	357±1.72 ^{Ga}
T2	108±0.35 ^{Ae}	265±3.74 ^{Ad}	473±3.32 ^{Ac}	570±3.26 ^{Ab}	660±3.53 ^{Aa}
T3	96± 0.72 ^{Ce}	185±1.15 ^{Cd}	299±2.05 ^{Cc}	386±1.63 ^{Cb}	531±2.51 ^{Ca}
T4	99± 0.65 ^{Ce}	161±2.23 ^{Dd}	273±1.89 ^{Dc}	352±2.70 ^{Db}	484±1.83 ^{Ea}
T5	87± 0.93 ^{De}	145 ± 1.88 ^{Ed}	235±3.66 ^{Fc}	274±3.62 ^{Eb}	493±2.49 ^{Da}
T6	86± 0.30 ^{eD}	124±0.75 ^{Fd}	243±2.83 ^{Ec}	255±1.30 ^{Fb}	478±1.84 ^{Fa}
T7	104±2.15 ^{Be}	253±1.94 ^{Bd}	429±3.41 ^{Bc}	496±2.49 ^{Bb}	553±2.18 ^{Ba}
$LSD_{0.5}$			4.12		

[1]Mean±SEM. T1 = *E. faecalis* only. T2 =: added *S. platensis* 2%, T3 = infected with *E. faecalis* + *S. platensis* 2%. T4 = infected with *E. faecalis* + probiotic. T5 *E. faecalis* + prebiotic. T6 = *E. faecalis* + oxytetracycline. T7 = Control. Different uppercase letters (A-G) refer to vertical statistical reading (among groups), lowercase letters (a-e) refer to horizontal statistical reading (among times) at P<0.05

It was observed that there was a significant decrease in the amount of feed intake by the chicks in group T_1 compared to the rest of the experiment groups in all the study weeks (P<0.05). While T_2 recorded a significant increase in the proportion of feed intake at P<0.05. The increase in the feed intake for group T_3 was more than for groups T_4, T_5, and T_6 when comparing the experimentally infected groups as shown in Table 4.

The results of the current study recorded a significant increase in the feed conversion rate in groups T_1 and T_6 (P<0.05) more than the other groups. Whereas the group experimentally infected with pathogenic bacteria T_3 recorded a significant decrease in F.C.R (P<0.05) when compared to the infected groups T_4 and T_5 as shown in Table 5.

Table 4. Effect of different levels of *Spirulina*, probiotic, prebiotic, and antibiotic with or without *E. faecalis* infection on weekly feed Intake (g) of broilers[1]

	Period				
Treatment	**Week1**	**Week2**	**Week3**	**Week4**	**Week5**
T1	150±1.72 ^{Ae}	213±4.20 ^{Ed}	342±2.58 ^{Gc}	442±6.23 ^{Gb}	678±7.83 ^{Ga}
T2	141±3.61 ^{BCDe}	296±2.56 ^{Cd}	591±7.11 ^{Bc}	823±5.10 ^{Bb}	1087±3.74 ^{Aa}
T3	139±2.55 ^{CDe}	280±5.62 ^{Dd}	557±2.40 ^{Cc}	671±5.27 ^{Fb}	975±4.39 ^{Da}
T4	151±2.49 ^{Ae}	322±3.86 ^{Bd}	518±2.16^{Dc}	676±5.75 ^{Eb}	1007±7.10 ^{Ca}
T5	128±3.09 ^{Ee}	278±4.55 ^{Dd}	411±5.51 ^{Fc}	781±2.64 ^{Cb}	962±5.62 ^{Ea}
T6	135±4.33 ^{De}	250±2.90 ^{Fd}	497±4.85 ^{Ec}	701±4.80 ^{Db}	946±5.06 ^{Fa}
T7	147±2.10 ^{ABe}	383±6.32 ^{Ad}	658±4.67 ^{Ac}	860±6.93 ^{Ab}	1070±3.82 ^{Ba}
$LSD_{0.5}$			7.28		

[1]Mean±SEM. T1 = *E. faecalis* only. T2 =: added *S. platensis* 2%, T3 = infected with *E. faecalis* + *S. platensis* 2%. T4 = infected with *E. faecalis* + probiotic. T5 *E. faecalis* + prebiotic. T6 = *E. faecalis* + oxytetracycline. T7 = Control. Different uppercase letters (A-G) refer to vertical statistical reading (among groups), lowercase letters (a-e) refer to horizontal statistical reading (among times) at P<0.05

Table 5. Effect of different levels of *Spirulina*, probiotic, prebiotic, and antibiotic with or without *E. faecalis* infection on feed conversion ratio (g:g) of broiler[1]

	Period				
Treatment	**Week1**	**Week2**	**Week3**	**Week4**	**Week5**
T1	1.84±0.10 ^{Ac}	1.80±0.03 ^{Bc}	2.02±0.04 ^{Aa}	1.93±0.08 ^{Aab}	1.90±0.05 ^{Bb}
T2	1.30±0.05 ^{Dc}	1.11±0.05 ^{Dd}	1.24±0.18 ^{Ec}	1.44±0.05 ^{Cb}	1.64±0.04 ^{Fa}
T3	1.43±0.06 ^{BCc}	1.52±0.17 ^{Cc}	1.86±0.11 ^{Ba}	1.73±0.10 ^{Bb}	1.83±0.7 ^{Cab}
T4	1.52±0.04 ^{Bc}	2.00±0.09 ^{Aab}	1.89±0.03 ^{Bb}	1.92±0.08 ^{Acb}	2.08±0.05 ^{Aa}
T5	1.56±0.04 ^{Bc}	1.91±0.03 ^{ABa}	1.74±0.03 ^{Cb}	1.94±0.05 ^{Aa}	1.95±0.02 ^{Ba}
T6	1.56±0.15 ^{Bb}	2.02±0.12 ^{Aa}	2.04±0.09 ^{Aa}	1.96±0.02 ^{Aa}	1.97±0.03 ^{ABa}
T7	1.42±0.05 ^{Cc}	1.50±0.05 ^{Cc}	1.53±0.06 ^{Dc}	1.73±0.13 ^{Bb}	1.93±0.09 ^{BCa}
$LSD_{0.5}$			0.112		

[1]Mean±SEM. T1 = E. faecalis only. T2 =: added *S. platensis* 2%, T3 = infected with *E. faecalis* + *S. platensis* 2%. T4 = infected with *E. faecalis* + probiotic. T5 *E. faecalis* + prebiotic. T6 = *E. faecalis* + oxytetracycline. T7 = Control. Different uppercase letters (A-G) refer to vertical statistical reading (among groups), lowercase letters (a-e) refer to horizontal statistical reading (among times) at P<0.05

DISCUSSION

Probiotics (*Lactobacillus acidophilus, L. casi, L. reuteri and Bacillus subtillis*) and prebiotics (contains extract of beta-glucans as antioxidants and a group of vitamins) work to stimulate the immune system of chicks and increase their ability to resist bacterial infections as well as stimulate the production of digestive enzymes in addition to containing vitamins that positively affect the health of birds (23).

Spirulina platensis is a multicellular, photosynthetic prokaryotic algae that contains a very high amount of proteins (55–70%) of the dry weight, vitamins and minerals (24, 25) which play an essential and important role in increasing the productive performance of broiler chickens (17, 26). It has been demonstrated by (27) that up to 10% of the total nutritional content from microalgae can be used safely as a partial diet substitute for traditional protein sources in poultry feeding. *Spirulina* in food increases the *Lactobacillus* population and enhances the absorption of vitamins (28).

The results of the current study showed that there was a significant increase at the level of P<0.05 in live body weight, as well as in the weekly weight gain for groups T_2 and T_3 (from the affected groups) during all weeks of the experiment compared with the control group. Group T_3 was among the groups experimentally infected with *E. faecalis* and treated with the addition of 2% of *S. platensis* powder to chicks drinking water, this addition caused a significant increase in the growth of chicks and improved health status due to its high protein ratio including important amino acids present in *Spirulina* and antioxidants content. In addition to improving the digestive ability of poultry, *Spirulina* also helped in strengthening the immune system and reduced the need for antibiotics, medications, and multivitamin supplementation, this is in agreement with the results of (29, 30) who indicated the possibility of improving chicken immunity response and substituting *Spirulina* instead of antibiotics. These improvements may be because of the synergetic effect of the chemical components (total phenolic and flavonoid contents and total antioxidant) found in *Spirulina*; these chemical constituents had antioxidant action (31, 32).

The current study showed a significant increase at the level of P<0.05 in feed intake in group T_2 compared to control group, while there was a significant increase and a lesser percentage in the groups T_3 and T_4 in comparison with experimentally infected groups. On the other hand, the study indicated a significant decrease in the feed conversion rate at P<0.05 in group T_2, as well as significant decrease in group T_3 for infected groups at P <0.05. These results are in agreement with (29, 33) who have also proven that adding *Spirulina* algae works to reduce food conversion ratio.

Overall, Adding of *Spirulina platensis* to drinking water at a percentage 2% led to a significant increase in the productive performance as well as improving the immunity

response and enhancement meat quality of broilers, this makes it a promising food addition in the poultry industry.

ACKNOWLEDGEMENTS

Authors would like to extend their deepest thanks to the members of the College of Veterinary Medicine, University of Baghdad for their support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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تحسين الأداء الإنتاجي لدجاج اللاحم عن طريق إضافة طحالب السبيرولينا ومقارنتها بالمعزز الحيوي والمغذيات الحيوية والأوكسي تيتراسايكلين

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير طحالب *Spirulina platensis* الممزوجة بمياه الشرب (2٪) على أداء إنتاج دجاج التسمين المصاب أو غير المصاب بالمكورات المعوية البرازية ومقارنتها بتأثير المعزز الحيوي والمغذيات الحيوية. اشتملت التجربة على 350 من افراخ دجاج التسمين عمرها يوم واحد (روز - 308) ، مقسمة عشوائياً إلى 7 مجموعات بواقع 50 فرخ لكل مجموعة لمدة 35 يوماً ، تم تقسيم المجموعات على النحو التالي: T1: أصابه تجريبية بالمكورات المعوية البرازية فقط ، T2: اضافة *S.platensis*فقط ، T3: اصابه بالمكورات المعوية البرازية ومعالج بـ 2 جم / لتر *S.platensis* ، T4: اصابه بالمكورات المعوية البرازية ويعالج بالبروبيوتيك ، T5: اصابه بالمكورات المعوية البرازية ويعالج بالبريبايوتك ، T6: اصابه بالمكورات المعوية البرازية ويعالج بـ أوكسي تتراسيكلين. T7: مجموعة السيطرة. أثبتت نتائج الدراسة الحالية أن *S.platensis* طحالب مضافة إلى مياه الشرب كان لها تأثير إيجابي على الأداء الإنتاجي لدجاج التسمين ، كما يمكن أن تكون *S. platensis* بديلاً جيدًا لبعض المضافات الغذائية مثل البروبيوتيك والبريبايوتكس والمضادات الحيوية وكذلك قدرته على تجنب الإصابة ببكتريا *Enterococcus faecalis*.

الكلمات المفتاحية: طحالب سبيرولينا، الاداء الانتاجي، دجاج اللاحم، المعزز الحيوي، المغذيات الحيوية