



Dietary Supplementation of *Spirulina platensis* as a Substitute for Antibiotics in Arab Chicken (*Gallus turcicus*)

N. A. Hasna, E. Widiastuti, I. Agusetyaningsih, E. C. Wulandari, R. Murwani, T. Yudiarti,
T. A. Sartono, S. Sugiharto, & H. I. Wahyuni*

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University
Jalan Prof. Sudarto, Tembalang, Semarang, 50275, Indonesia

*Corresponding author: hihannyiw123@gmail.com

(Received 19-10-2023; Revised 01-12-2023; Accepted 20-12-2023)

ABSTRACT

This study was conducted to determine the effect of adding *Spirulina platensis* to replace zinc bacitracin on performance, egg quality, blood profile, total gut bacteria, and liver histopathology of local indigenous Arab chicken (*Gallus turcicus*). One hundred and eight 28-week-old laying hens were distributed randomly to three treatments: T0 (control diet), T1 (T0 + 1% *S. platensis*), and T2 (T0 + 0.04% zinc bacitracin). The treatment was applied for 49 days. *S. platensis* and zinc bacitracin decreased feed intake ($p < 0.05$), but the egg mass had no significant effect; this provides a good improvement in feed conversion ratio ($p < 0.05$). *S. platensis* helped maintain persistent egg production ($p < 0.05$). *S. platensis* and zinc bacitracin provided the best results on haugh unit ($p < 0.05$). *S. platensis* increased the yolk score ($p < 0.05$). Zinc bacitracin decreased erythrocytes ($p < 0.05$) but was not significantly different from *S. platensis*. *S. platensis* and zinc bacitracin increased mean corpuscular volume (MCV) ($p < 0.05$). Blood chemical profile and total gut bacteria were not affected by the treatments. *S. platensis* was the best at maintaining liver's health ($p < 0.05$). This research concludes that *S. platensis* can efficiently optimize feed consumption, enhance performance, maintain egg quality, and protect the liver damage of Arab chicken. Therefore, *S. platensis* could be considered to replace the use of antibiotics.

Keywords: blood profile; egg quality; liver histopathology; *Spirulina platensis*; zinc bacitracin

INTRODUCTION

Arab chicken (*Gallus turcicus*) has been intensively reared for egg production in Indonesia. Arab chicken is a crossbreed between the Braekels male chicken from Belgium and a local female (Puteri *et al.*, 2020). Arab chicken was developed in 1995 in East Java. It was named 'Arab chicken' because the white feathers cover the head like wearing Arab clothing, specifically a hijab (Tamzil & Indarsih, 2022). Arab chicken hens have high egg production and robust immunity. They begin producing eggs at 18–22 weeks of age and attain peak production at 32 weeks (Indra *et al.*, 2013). The sharp decrease in egg production after peak production is the major challenge in raising Arab chicken hens.

Antibiotic growth promoters (AGPs) added to laying hen's diet on a regular basis would assist in preserving their health and increasing nutrient absorption, resulting in optimal egg production following peak production. However, the negative effects of incorrect and continuous usage of AGPs may increase the growth of antibiotic-resistant bacteria in animal products consumed by humans (Samreen *et al.*, 2021). An alternative to AGPs is incorporating an organic additive such as *Spirulina platensis* into the feed, as it would also serve as a natural antioxidant, anti-inflammatory agent, and

immunomodulatory (Selim *et al.*, 2018; Farag *et al.*, 2016). With the natural active ingredients in *S. platensis*, livestock products are expected to be free from antibiotic residue and support animal welfare by not causing antibiotic resistance in laying hens (Nannoni *et al.*, 2023).

Several studies reported the role of *S. platensis* as a feed additive in laying hens that enhanced its performance and egg quality, such as increased egg production percentage (Curabay *et al.*, 2021), improved feed conversion ratio (FCR) (Selim *et al.*, 2018), increased retinol content in the yolk (Rey *et al.*, 2021), and the egg yolk score (Vaz *et al.*, 2016). There is an increase in *Lactobacillus* population in the cecal contents of chickens given additional *S. platensis* (Park *et al.*, 2018). *S. platensis* efficiently improves blood hematological parameters in chickens subjected to heat stress, exhibit lower levels of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT), along with increased superoxide dismutase activity (Abbas *et al.*, 2022; Tufarelli *et al.*, 2021). *S. platensis* increased the total leukocyte count, leading to enhanced phagocytic activity to strengthen the chicken's immune system (Mariey *et al.*, 2014) and improve the kidney and liver tissues of broiler chickens damaged by cadmium chloride (Berbesh *et al.*, 2022).

There is limited research on the use of *S. platensis* in local Indonesian chickens, especially in Arab chicken. Therefore, this study is important to explore an alternative to AGP without causing negative effects on Arab chicken's health. This research aims to assess the use of *S. platensis* as a replacement for zinc bacitracin antibiotic in terms of performance, egg quality, blood profile, liver histopathology, and total gut bacteria in Arab chicken.

MATERIALS AND METHODS

Experimental Animals, Housing, and Management

A total of 108 Arab chicken, aged 28 weeks with an average body weight of $1,088 \pm 56.52$ g, were sourced from a commercial farm and used for this study. The hens were selected based on their age and weight to ensure uniformity. The study was conducted in battery cages with a lighting duration of 15 hours daily. The hens were manually fed twice daily (morning and evening) and had *ad libitum* access to drinking water. The experiment was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang with approval number 59-10/A-23/KEP-FPP.

Experimental Design and Treatment Diet

The hens were randomly divided into three treatment groups with six replicates, resulting in 18 experimental units. Each experimental unit contained 6 hens, and each battery cage housed 2 hens. The treatments applied in the study included T0 (control group), T1 (T0 + 1% *S. platensis*), and T2 (T0 + 0.04% zinc bacitracin). The treatment diet was applied for 49 days. Composition and nutritional content of *S. platensis* powder obtained from PT. Polaris and its proximate analysis are shown in Table 1, while the control diet is shown in Table 2. Zinc bacitracin was obtained from PT. Batako Indonesia, Magelang.

Variables Measured

Feed intake, hen day production (HDP), egg mass, and FCR were recorded daily. The percentage of the decreased HDP was calculated by: $(\text{HDP at day 49} - \text{HDP at day 1}) / \text{HDP at day 1} \times 100\%$.

One egg was collected randomly from each experimental unit at the last three days of the research (days 47, 48, and 49). The albumen index and yolk index were calculated according to Curabay *et al.* (2021). Haugh unit was determined following the method by Stadelman & Cotterill (1995). Yolk color was measured using a yolk color fan with 15 color scales: Scale 1 shows a bright yellow yolk color while scale 15 shows a more reddish-orange yolk color (Kaewtapee & Supratak, 2021). Shell thickness was measured using a screw micrometer.

One hen was randomly selected from each experimental unit to obtain a blood sample. The blood sample was collected in 3 mL from the brachial vein using a syringe. A 1.5 mL blood sample was placed in

a vacutainer with EDTA as an anticoagulant for blood profile analysis using an automatic hematology analyzer (PT. Alkesindo Nusantara, Jakarta, Indonesia). Blood profile analysis included erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), number of platelets, leukocyte, and lymphocyte.

Another part of the blood sample was placed in a vacutainer without anticoagulant and centrifuged at 3000 rpm for 15 minutes to obtain the serum (Darmawan *et al.*, 2019). Total protein was analyzed using the biuret method, which is the reaction of proteins with copper ions in an alkaline medium, forming a blue or purple color (Murmu *et al.*, 2021). Albumin was analyzed using the bromocresol green method (Phoonsawat *et al.*, 2021). Globulin data were obtained by subtracting the albumin value from the total protein value (An *et al.*, 2016). SGPT and SGOT were analyzed using the IFCC method to measure enzyme activity (Sijabat *et al.*, 2023).

The laying hens from which blood samples were taken were decapitated to get the liver as well as the digesta from ileum and cecum. The liver was sampled for histopathological analysis, and the digesta samples were analyzed for total *Coliform* and lactic acid bacteria.

Table 1. Composition of *Spirulina platensis* powder and proximate analysis

Nutrients	Label composition ^{*)}	Proximate analysis
Moisture (%)	-	6.91
Crude protein (%)	60	57.68
Crude fiber (%)	6	1.35
Crude fat (%)	-	2.04
Ash (%)	-	4.68
Linoleic acid (mg/10 g)	130	-
R-Linoleic acid (mg/10 g)	135	-
Isoleucine (mg/10 g)	350	-
Lysine (mg/10 g)	290	-
Phenylalanine (mg/10 g)	280	-
Tryptophane (mg/10 g)	90	-
Leucine (mg/10 g)	540	-
Methionine (mg/10 g)	140	-
Threonine (mg/10 g)	320	-
Valine (mg/10 g)	400	-
Vitamin B1 (mg)	0.45	-
Vitamin B2 (mg)	0.4	-
Vitamin B3 (mg)	1.45	-
Vitamin B6 (mcg)	80	-
Vitamin B12 (mcg)	32	-
Vitamin A (IU)	23000	-
Vitamin E (IU)	4	-
Pantothenic acid (mg/100 g)	4	-
Folic acid ($\mu\text{g}/100$ g)	100	-
Phycocyanin (%)	20	-
Chlorophyll (%)	1.5	-
β -carotenoids (%)	0.15	-
Polysaccharide (g/100 g)	0.4	-

Note: *) As stated on the labelling of the product.

Liver tissue samples measuring 1x1 cm were collected and placed in a 10% phosphate buffer formalin solution for fixation within 24 hours, embedded in paraffin, and cut 5 µm (Rashidi *et al.*, 2020). Sections were stained with Hematoxylin-Eosin before microscopic examination. Liver lesion scores were classified into four levels: 1= no vacuolization, 2= less than 50% of hepatocytes have varying sizes of vacuoles, 3= more than 50% of hepatocytes have varying sizes of vacuoles or small vacuolization, 4= distribution of vacuolization in medium to large vacuoles (Karimirad *et al.*, 2020).

Samples of ileum and cecum digesta were collected and placed in sterile containers. Total bacteria were analyzed based on Sugiharto *et al.* (2018), MacConkey agar medium was used to observe *Coliform* bacteria growth. It was incubated aerobically at 38 °C for 24 hours, and *Coliform* growth was indicated by a red color.

Mann Rogosa Sharpe medium was used to grow lactic acid bacteria (LAB). It was incubated anaerobically at 38 °C for 48 hours, and white bacterial colonies indicated LAB growth. After incubation, the colonies of each type of bacteria were counted in log cfu/g.

Statistical Analysis

Data were processed using SPSS software version 25. Parametric data (performance, blood profile, and total gut bacteria) were analyzed using one-way analysis of variance (ANOVA) with a significance level of 5%. Means with significant differences in results were further analyzed using Duncan’s multiple range test. Non-parametric data, such as yolk color and liver histopathology scores, were analyzed using the Kruskal-Wallis Test.

Table 2. Composition and nutrient content of control diet

Items	Composition (%)
Feed ingredients	
Corn	43
Rice bran	28.7
Meat bone meal	13
Soybean meal	10
CaCO ₃	3
Grit	2
Premix ^{*)}	0.3
The analyzed nutritional contents	
Metabolizable energy (ME) (kcal/kg ^{**)}	2,888
Crude protein	19.86
Crude fiber	5.61
Crude fat	3.76
Ash	9.53
Moisture	11.09
Calcium ^{***)}	2.58
Phosphorus ^{***)}	1.05

Note: *) Premix Composition (per 10 kg): vitamin A 12.000.000 IU, vitamin D-3 2.000.000 IU, vitamin E 8.000 IU, vitamin K₃ 2.000 mg, vitamin B₁ 2.000 mg, vitamin B₂ 5.000 mg, vitamin B₆ 500 mg, vitamin B₁₂ 12.000 µg, vitamin C 25.000 mg, calcium-D-pantothenate 6.000 mg, niacin 40.000 mg, cholin chloride 10.000 mg, methionine 30.000 mg, lysine 30.000 mg, Mn 120.000 mg, Fe 20.00 mg, iodine 200 mg, Zn 100.000 mg, Co 200 mg, Cu 4.000 mg, and santoquin (antioxidant) 10.000 mg.
 **) ME was calculated using the Bolton formula (1967) as cited by Sugiharto *et al.* (2018): 40.81 × (0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5).
 ***) NRC (1994).

RESULTS

Performance

The performance data in Table 3 showed that the addition of *S. platensis* and zinc bacitracin significantly reduced feed intake (p<0.05) while maintaining the same egg mass compared to the control diet. Hence, it significantly (p<0.05) improved the feed conversion ratio for both treatments (*S. platensis* and zinc bacitracin). Additionally, *S. platensis* helped maintain persistent egg production, as shown by the significantly lowest value of the decreasing HDP percentage (p<0.05).

Egg Quality

Egg quality data are shown in Table 4; a diet with zinc bacitracin significantly improved haugh unit (p<0.05) but was not significantly different from the diet with *S. platensis*. A diet with *S. platensis* showed the best yolk color values (p<0.05).

Blood Profile

The blood profile data in Table 5 showed that the zinc bacitracin significantly reduced erythrocyte count (p<0.05), and both *S. platensis* and zinc bacitracin increased MCV (p<0.05). The addition of *S. platensis* and zinc bacitracin resulted in similar values for hemoglobin, hematocrit, MCH, MCHC, platelets,

Table 3. Feed intakes, HDP, egg mass, and FCR of Arab chicken fed diets supplemented with *Spirulina platensis* or zinc bacitracin

Variables	Treatments			SEM	p-value
	T0	T1	T2		
Feed intake (g/bird/day)	63.87 ^a	55.59 ^b	56.98 ^b	1.152	0.001
Initial HDP (%)	58.33	59.72	55.55	1.424	0.506
End HDP (%)	46.03 ^b	57.54 ^a	54.76 ^{ab}	2.128	0.060
Decreasing HDP (%)	25.73 ^a	11.79 ^b	17.71 ^{ab}	2.404	0.048
Egg mass (g/bird/day)	23.19	24.65	25.64	0.699	0.379
FCR	2.74 ^a	2.31 ^b	2.26 ^b	0.079	0.009

Note: ^{ab} Means in the same row with different superscripts differ significantly (p<0.05). T0= Control diet, T1= T0 + 1% *S. platensis*, T2= T0 + 0.04% zinc bacitracin. SEM= standard error of the means, HDP= hen day production, FCR= feed conversion ratio.

leukocytes, and lymphocytes with the control diet. All blood biochemical parameters of Arab chicken were not affected by the treatments.

Liver Histopathology

Data on the liver histopathology of Arab chicken in Table 5 showed that adding *S. platensis* was the best in maintaining the liver's health ($p < 0.05$).

Total Gut Bacteria Population

The data on the total gut bacteria population in Arab chicken are presented in Table 6. The treatments

did not affect the total Coliform and LAB bacteria in the ileum and cecum.

DISCUSSION

The addition of *S. platensis* to the diet lowers the feed intake. It was likely due to the darker color of the feed caused by Spirulina. This coloration affects the sensitivity of chickens on feed consumption (Ahmad *et al.*, 2022; Elahi *et al.*, 2019). Spirulina, as a protein source, contains 57.66% crude protein (Zeweil *et al.*, 2016), and it reduces feed consumption. However, giving 1% *S. platensis* for 7 weeks resulted in the highest production at the end of the study. It helped maintain

Table 4. Egg quality of Arab chicken fed diets with *Spirulina platensis* or zinc bacitracin

Variables	Treatments			SEM	p-value
	T0	T1	T2		
Albumen index	0.071	0.076	0.081	0.001	0.115
Yolk index	0.43	0.45	0.45	0.005	0.309
Haugh unit	90.77 ^b	95.29 ^a	96.46 ^a	0.870	0.009
Yolk score	9.16 ^b	11.66 ^a	8.66 ^b	0.335	<0.001
Shell thickness (mm)	0.52	0.52	0.53	0.003	0.904

Note: ^{a,b} Means in the same row with different superscripts differ significantly ($p < 0.05$). T0= Control diet, T1= T0 + 1% *S. platensis*, T2= T0 + 0.04% zinc bacitracin. SEM= standard error of the means.

Table 5. Blood profile and liver histopathology score of Arab chicken fed diets supplemented with *Spirulina platensis* or zinc bacitracin

Variables	Treatments			SEM	p-value
	T0	T1	T2		
Erythrocytes ($10^{12}/L$)	2.76 ^a	2.43 ^{ab}	2.29 ^b	0.079	0.037
Hemoglobin (g/dL)	15.91	15.53	15.36	0.289	0.753
Hematocrit (%)	33.63	31.95	30.25	0.841	0.274
MCV (fl)	122.10 ^b	131.20 ^a	134.10 ^a	1.614	0.001
MCH (pg)	63.00	63.85	67.43	1.646	0.535
MCHC (g/dL)	47.45	48.81	50.93	0.854	0.257
Platelets ($10^3/mm^3$)	46.16	40.66	43.16	1.475	0.332
Leukocytes ($10^3/mm^3$)	95.31	88.01	90.21	1.693	0.202
Lymphocytes (%)	74.66	77.266	76.81	1.071	0.599
Total protein (g/dL)	5.25	5.09	5.15	0.235	0.965
Albumin (g/dL)	1.75	1.87	1.87	0.038	0.341
Globulin (g/dL)	3.50	3.22	3.27	0.230	0.884
SGPT (U/L)	3.98	4.65	3.57	0.471	0.674
SGOT (U/L)	221.47	194.05	190.19	13.691	0.625
Liver histopathology score	3.66 ^a	1.66 ^b	3.00 ^a	0.249	0.004

Note: ^{a,b} Means in the same row with different superscripts differ significantly ($p < 0.05$). T0= Control diet, T1= T0 + 1% *S. platensis*, T2= T0 + 0.04% zinc bacitracin. SEM= standard error of the means, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, SGPT= serum glutamic pyruvic transaminase, SGOT= serum glutamic oxaloacetic transaminase.

Table 6. Total gut bacterial population of Arab chicken fed diets supplemented with *Spirulina platensis* or zinc bacitracin

Variables	Treatments			SEM	p-value
	T0	T1	T2		
Ileum (log cfu/g)					
Coliform	1.99	1.83	2.16	0.092	0.349
Lactic acid bacteria	2.09	2.17	2.43	0.288	0.895
Cecum (log cfu/g)					
Coliform	3.04	3.01	3.10	0.043	0.744
Lactic acid bacteria	1.98	2.61	2.14	0.225	0.524

Note: T0= Control diet, T1= T0 + 1% *S. platensis*, T2= T0 + 0.04% zinc bacitracin. SEM= standard error of the means.

persistence in egg production during the decline after the peak production period in Arab chicken. *S. platensis* supplementation in this study increased the number of ovarian follicles or protect it from shrinkage due to the essential amino acids and active compounds such as polysaccharides, unsaturated fatty acids, and carotenoids, which play a physiological role in enhancing antioxidant activity and immunity (Korany *et al.*, 2019; Ma *et al.*, 2020). These findings were consistent with Ismail *et al.* (2023) and Selim *et al.* (2018), who showed that a laying chicken's diet supplemented with *S. platensis* produced more eggs than those fed a diet without *S. platensis*. However, *S. platensis* and zinc bacitracin had no differences in egg mass of Arab chicken. This is in line with the study by Curabay *et al.* (2021), which found that supplementing *S. platensis* to Lohmann LSL Classic chickens did not significantly affect egg mass. Both *S. platensis* and zinc bacitracin supplementation improved the FCR values in Arab chicken. The addition of *S. platensis* and zinc bacitracin helped maintain egg production levels and enhance the FCR values of laying hens (Selim *et al.*, 2018; Feng & Zhongsheng, 2019).

The addition of both *S. platensis* and zinc bacitracin improved the haugh unit in eggs. Haugh unit is influenced by the protein content in the diet. Protein derived from the digestion process in the small intestine are transported by the blood as amino acids to the liver for protein synthesis. The resulting protein is then carried by the blood to the oviduct for albumen production (Sadr *et al.*, 2023). *S. platensis* demonstrates similar results to the zinc bacitracin treatment, likely because *S. platensis* has good quality and quantity of protein. The essential amino acids found in *S. platensis* are methionine and histidine (Tessier *et al.*, 2021). The protein affects the components of albumen. One of the components of albumen is ovomucin, and it has the function of binding albumen fluid into a gel form (Sunarno *et al.*, 2023). Regarding yolk color, *S. platensis* treatment yielded the highest scores with a more intense yellow color. *S. platensis* contains xanthophylls and beta-carotene, which enhance yolk pigmentation (Omri *et al.*, 2019). However, it did not significantly affect shell thickness (Curabay *et al.*, 2021).

A diet with *S. platensis* addition helped maintain the number of erythrocytes compared to zinc bacitracin. The compound phycocyanin found in *S. platensis* stimulates the production of the erythropoietin hormone, which plays a role in erythropoiesis (Sarker *et al.*, 2022). Phycocyanin provides a signal to stimulate erythropoietin hormone produced by the kidneys, which then produces proerythroblasts in the bone marrow. Erythroblasts undergo maturation processes and become reticulocytes, which then circulate throughout the body as erythrocytes. Meanwhile, MCV value is influenced by erythrocytes and is more sensitive in indicating the possibility of iron deficiency (Suzana *et al.*, 2017). *S. platensis* is rich in important minerals, one of which is iron, which is used in erythropoiesis (Al-Otaibi *et al.*, 2022). Treatment with *S. platensis* tends to have a lower total protein concentration. The lowering effect in total protein would be due to the presence of antioxidant compounds in *S. platensis*, such as steroids, phenols, flavonoids (Agustini *et al.*, 2015), polysaccharides, vitamin B12, vitamin K, vitamin A, and vitamin E (Rey *et al.*, 2021). Additional *S. platensis* reduced the concentration of SGPT and SGOT released by the liver into the bloodstream, indicating its role on hepatoprotective activity (Tufarelli *et al.*, 2021).

The liver's histopathology showed damage in the control group, with several hepatocytes vacuolization leading to cell death (necrosis), characterized by the absence of cell nuclei (Figure 1). Treatment with *S. platensis* depicted mild hepatocyte vacuolization, close to normal. Normal hepatocytes had a round cell shape and visible nucleus (Figure 2). This is because *S. platensis* acts as a hepatoprotector and immunostimulant due to its content of vitamins, β -carotene, and the phycocyanin pigment, which protects the liver during detoxification processes and enhances the immune system (Sharoud, 2015). *S. platensis* also contains enzymes like superoxide dismutase (SOD) and catalase, which act as an antioxidant (Kumar *et al.*, 2022). These compounds were transported from the bloodstream to the liver, so the addition of *S. platensis* prevented liver tissue damage caused by free radicals. SOD will catalyze the dismutation of superoxide anion into H_2O_2 and O_2 . The abundant production of H_2O_2 was toxic; therefore,

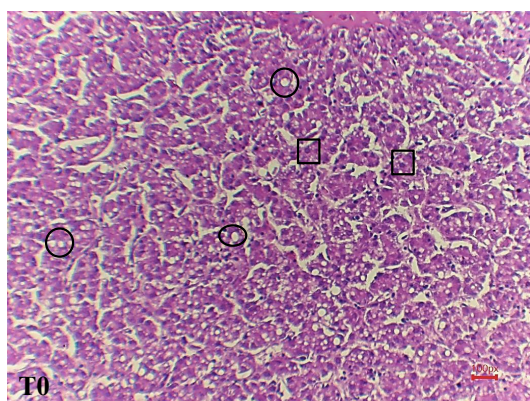


Figure 1. Liver histopathology of Arab chicken fed control diet (T0) at 100 \times magnification. Normal hepatocyte (black square) and hepatocyte vacuolization (black circle).

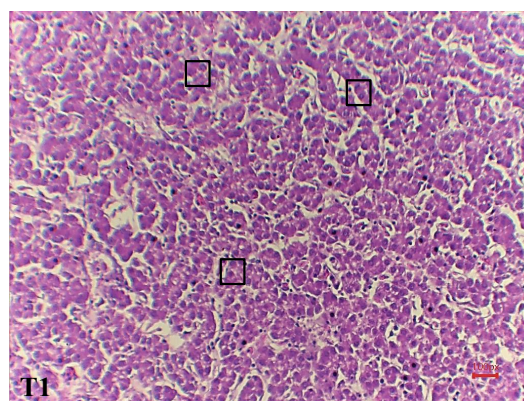


Figure 2. Liver histopathology of Arab chicken fed *Spirulina platensis* diet (T1) at 100 \times magnification. Hepatocyte vacuolization (black circle) and normal hepatocyte (black square).

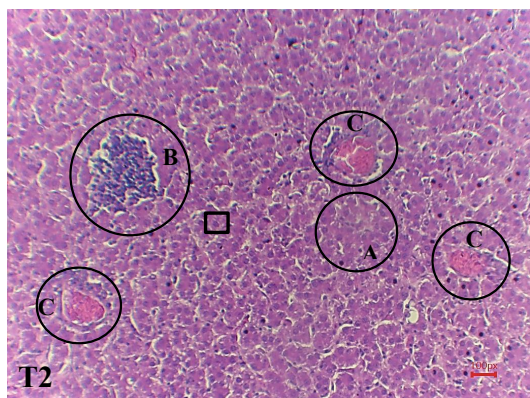


Figure 3. Liver histopathology of Arab chicken fed zinc bacitracin diet (T2) at 100× magnification. Normal hepatocyte (black square), early stage of necrotic cells (A), lymphocytes accumulation (B), and congestion (C).

detoxification is necessary with the catalase enzyme, which catalyzes H_2O_2 into H_2O and O_2 (Zhou *et al.*, 2019). Treatment with zinc bacitracin also displayed liver cell damage, such as necrosis, congestion, and lymphocyte accumulation (Figure 3). This was following the finding of Attia *et al.* (2015) that showed sinusoidal congestion in rabbits' livers due to continuous exposure to toxic substances from zinc bacitracin. The liver would impact the metabolism processes, affecting the chicken's productivity.

The results of the total gut bacteria from this study are different from the research by Joya *et al.* (2021) and Sugiharto *et al.* (2018) stated that the administration of *S. platensis* suppressed coliform bacteria in the chicken's intestines. Alkaloid compounds, lipopolysaccharides, and cyclic peptides in algae have an antibacterial activity; therefore, *S. platensis* would reduce pathogenic bacteria in the chicken's digestive tract (El-Sheekh *et al.*, 2014).

The limitation of this research is the high cost of *S. platensis*. Therefore, it is crucial to use lower doses to avoid burdening production costs. A practical application that needs to be carried out is an economic analysis to assess the economic feasibility of using *S. platensis*. Besides, the number of replications results in a relatively large error; hence, further research with a larger number of replications is necessary. Practical application in this regard involves using a larger population of Arab chicken.

Overall, this study indicates that the administration of *S. platensis* may not enhance nutrient absorption, but *S. platensis* is rich in amino acids, minerals, vitamins, and antioxidant compounds that are easily absorbed by the digestive system of the laying hen. Consequently, these nutrients are used in metabolism processes such as protecting liver tissue from damage and optimizing its metabolic processes, resulting in egg production persistence and good egg quality as well.

CONCLUSION

In conclusion, *S. platensis* can efficiently optimize feed consumption, enhance performance, maintain egg

quality, and protect the liver damage of Arab chicken. Therefore, *S. platensis* could be considered to replace the use of antibiotics.

CONFLICT OF INTEREST

We declare no conflict of interest with any personal, other relationship or financial, with other organizations or people related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

The authors would like to express gratitude to the research team, laboratory technicians, and financial support from the Faculty of Animal and Agricultural Sciences, Diponegoro University.

REFERENCES

- Abbas, A. O., A. A. Alaql, G. M. K. Mehaisen, & N. N. Kamel. 2022. Effect of dietary blue-green microalgae inclusion as a replacement to soybean meal on laying hens performance, egg quality, plasma metabolites, and hematology. *Animals* 12:2816. <https://doi.org/10.3390/ani12202816>
- Agustini, T. W., M. Suzery, D. Sutrisnanto, W. F. Ma'ruf, & Hadiyanto. 2015. Comparative study of bioactive substances extracted from fresh and dried *Spirulina sp.* *Procedia Environ. Sci.* 23:282–289. <https://doi.org/10.1016/j.proenv.2015.01.042>
- Ahmad, I., M. Ullah, M. Alkafafy, N. Ahmed, S. F. Mahmoud, K. Sohail, H. Ullah, W. M. Ghoneem, M. M. Ahmed, & S. Sayed. 2022. Identification of the economics, composition, and supplementation of maggot meal in broiler chicken. *Saudi J. Biol. Sci.* 29:103277. <https://doi.org/10.1016/j.sjbs.2022.03.027>
- Al-Otaibi, M. I., H. A. Abdellatif, A. K. Al-Huwail, A. O. Abbas, G. M. Mehaisen, & E. S. Moustafa. 2022. Hypocholesterolemic, antioxidative, and anti-inflammatory effects of dietary *Spirulina platensis* supplementation on laying hens exposed to cyclic heat stress. *Animals* 12:2759. <https://doi.org/10.3390/ani12202759>
- An, H. M., Y. L. Tan, S. P. Tan, J. Shi, Z. R. Wang, F. D. Yang, X. F. Huang, J. C. Soars, T. R. Kosten, & X. Y. Zhang. 2016. Smoking and serum lipid profiles in schizophrenia. *Neurosci. Bull.* 32:383–388. <https://doi.org/10.1007/s12264-016-0022-0>
- Attia, Y. A., R. S. Hamed, A. E. Abd El-Hamid, M. A. Al-Harathi, H. A. Shahba, & F. Bovera. 2015. Performance, blood profile, carcass and meat traits and tissue morphology in growing rabbits fed mannanoligosaccharides and zinc-bacitracin continuously or intermittently. *Anim. Sci. Pap. Rep.* 33:85–101.
- Berbesh, S., R. El-Shawarby, E. El-Shewy, S. El-Sheshtawy, & S. Elshafae. 2022. Ameliorative effect of *Spirulina platensis* against cadmium toxicity in broiler chickens. *Benha Vet. Med. J.* 42:51–55. <https://doi.org/10.21608/bvmj.2022.111546.1490>
- Curabay, B., B. Sevim, Y. Cufadar, & T. Ayasan. 2021. Effects of adding *Spirulina plantesis* to laying hens rations on performance, egg quality, and some blood parameters. *J. Hellenic Vet. Med. Soc.* 72:2945–2952.
- Darmawan, M. A., Y. Y. Suranindyah, & D. T. Widayati. 2019. The correlation between blood metabolic and reproductive performance on the holstein-friesian crossbred dairy cows. *IOP Conf. Ser. Earth Environ. Sci.* 387:012023. <https://doi.org/10.1088/1755-1315/387/1/012023>

- Elahi, U., Y. Ma, S. Wu, J. Wang, H. Zhang, & G. Qi. 2019. Growth performance carcass characteristic, meat quality and serum profile of broiler chicks fed on housefly maggot meal as a replacement of soybean meal. *J. Anim. Physiol. Anim. Nutr.* 4:1075–1084. <https://doi.org/10.1111/jpn.13265>
- El-Sheekh, M. M., S. M. Daboor, M. A. Swelim, & S. Mohamed. 2014. Production and characterization of antimicrobial active substance from *Spirulina platensis*. *Iran J. Microbiol.* 6:112–119. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4281658/pdf/IJM-6-112.pdf>
- Farag, M. R., M. Alagawany, M. E. A. El-Hac, & K. Dhama. 2016. Nutritional and healthical aspects of spirulina (*Arthrospira*) for poultry. *Intern. J. Pharmacol.* 12:36–51. <https://doi.org/10.3923/ijp.2016.36.51>
- Feng, Z. & X. Zhongsheng. 2019. Effects of Dietary Fructo-Oligosaccharides on Laying Performance and Serum Biochemical Parameters of Yellow Broiler Breeder Hens. In *E3S Web of Conferences*, 131, p. 01081. EDP Sciences. <https://doi.org/10.1051/e3sconf/201913101081>
- Ismail, F., K. Sherif, Y. Rizk, M. Hassan, A. Mekawy, & K. Mahrose. 2023. Dietary supplementation of spirulina and canthaxanthin boosts laying performance, lipid profile in blood and egg yolk, hatchability, and semen quality of chickens. *J. Anim. Physiol. Anim. Nutr.* 107:650–658. <https://doi.org/10.1111/jpn.13729>
- Indra, G. K., Achmanu, & A. Nurgartiningih. 2013. Performance production of arab chicken (*Gallus turcicus*) based on feather color. *Ternak Tropika* 14:8–14.
- Joya, M., O. Ashayerizadeh, & B. Dastar. 2021. Effects of *Spirulina* (*Arthrospira*) *platensis* and *Bacillus subtilis* PB6 on growth performance, intestinal microbiota and morphology, and serum parameters in broiler chickens. *Anim. Prod. Sci.* 61:390–398. <https://doi.org/10.1071/AN20218>
- Karimirad, R., H. Khosravinis, & B. P. Kavan. 2020. Effect of differeny feed physical forms (pellet, crumble mash) on the performance and liver health in broiler chicken with and without carbon tetrachloride challenge. *J. Anim. Feed Sci.* 29:59–66. <https://doi.org/10.22358/jafs/118818/2020>
- Kaewtapee, C. & A. Supratak. 2021. Yolk color measurement using image processing and deep learning. *IOP Conf. Ser. Earth Environ. Sci.* 686:012054. <https://doi.org/10.1088/1755-1315/686/1/012054>
- Korany, R. M. S., K. S. Ahmed, H. A. El-Halawany, & K. A. Ahmed. 2019. Pathological and immunohistochemical studies on the ameliorating effect of *Spirulina platensis* against arsenic induced reproductive toxicity in female albino rats. *Int. J. Vet. Sci.* 8:113–119. <http://www.ijvets.com/pdf-files/Volume-8-no-2-2019/113-119.pdf>
- Kumar, A. D. Ramamoorthy, D. K. Verma, A. Kumar, N. Kumar, K. R. Kanak, B. M. Marwein, & K. Mohan. 2022. Antioxidant and phytonutrient activities of *Spirulina platensis*. *Energy Nexus.* 6:100070. <https://doi.org/10.1016/j.nexus.2022.100070>
- Ma, W. Q., H. Z. Cheng, D. H. Zhao, J. Yang, S. B. Wang, H. Z. Wu, M. Y. Lu, L. Xu, & G. J. Liu. 2020. Effects of dietary *Enteromorpha* powder supplementation on productive performance, egg quality and antioxidant performance during the late laying period in Zi geese. *Poult. Sci.* 99:1062–1068. <https://doi.org/10.1016/j.psj.2019.10.003>
- Marieb, Y. A., H. R. Samak, H. Abou-Khashba, M. Sayed, & A. Abou-Zeid. 2014. Effect of using *Spirulina platensis* algae as feed additives for poultry diets: 2 productive performance of broiler. *Egypt. Poult. Sci.* 34:245–258.
- Murmu, A. L., R. K. Verma, S. K. Yadav, S. Barik, S. K. Maurya, & S. Kumar. 2021. Effects of different meteorological variables on blood biochemical parameters in black Bengal goats. *J. Entomol. Zool. Stud.* 9:1887–1895. <https://doi.org/10.22271/j.ento.2021.v9.i1aa.8407>
- Nannoni, E., G. Martelli, M. Scozzoli, S. Belperio, G. Buonaiuto, N. I. Vannetti, E. Truzzi, E. Rossi, S. Benvenuti, & L. Sardi. 2023. Effects of lavender essential oil inhalation on the welfare and meat quality of fattening heavy pigs intended for parma ham production. *Animals* 13:2967. <https://doi.org/10.3390/ani13182967>
- National Research Council. 1994. *Nutrient Requirements of Poultry: Ninth Revised Edition*. Washington, DC. The National Academies Press. <https://doi.org/10.17226/2114>
- Omri, B., M. Amraoui, A. Tarek, M. Lucarini, A. Durazzo, N. Cicero, A. Santini, & M. Kamoun. 2019. *Arthrospira platensis* (*Spirulina*) supplementation on laying hens' performance: Eggs physical, chemical, and sensorial qualities. *Foods* 8:386. <https://doi.org/10.3390/foods8090386>
- Park, J. H., S. I. Lee, & I. H. Kim. 2018. Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poult. Sci.* 97:2451–2459. <https://doi.org/10.3382/ps/pey093>
- Phoonsawat, K., K. Khachornsakkul, N. Ratnarathorn, C. S. Henry, & W. Dungchai, W. 2021. Distance-based paper device for a naked-eye albumin-to-alkaline phosphatase ratio assay. *ACS Sens.* 6:3047–3055. <https://doi.org/10.1021/acssensors.1c01058>
- Puteri, N. I., Gushairiyanto, & Depison. 2020. Growth patterns, body weight, and morphometric of KUB chicken, Sentul chicken and Arab chicken. *Bulletin Animal Science* 44:67–72. <https://doi.org/10.21059/buletinpeternak.v44i3.57016>
- Rashidi, N., A. Khatibjoo, K. Taherpour, M. Akbari-Gharaei, & H. Shirzadi. 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. *Poult. Sci.* 99:5896–5906. <https://doi.org/10.1016/j.psj.2020.08.034>
- Rey, A. I., A. de-Cara, A. Rebolé, & I. Arija. 2021. Short-term spirulina (*Spirulina platensis*) supplementation and laying hen strain effects on eggs' lipid profile and stability. *Animals* 11:1944. <https://doi.org/10.3390/ani11071944>
- Sadr, S., N. Lotfalizadeh, S. A. Ghafouri, M. Delrobaei, N. Komeili, & A. Hajjafari. 2023. Nanotechnology innovations for increasing the productivity of poultry and the prospective of nanobiosensors. *Vet. Med. Sci.* 9:2118–2131. <https://doi.org/10.1002/vms3.1193>
- Samreen, I. Ahmad, H. A. Malak, & H. H. Abulreesh. 2021. Environmental antimicrobial resistance and its drivers: a potential threat to public health. *J. Glob. Antimicrob. Resist.* 27:101–111. <https://doi.org/10.1016/j.jgar.2021.08.001>
- Sarker, M. S., K. Rafiq, M. M. Rahman, K. K. I. Khalil, M. S. Islam, & M. S. Islam. 2022. Effects of spirulina (*Spirulina platensis*) on production, hematological parameters and lipid profile in layers. *Agriculture Livestock Fisheries* 9:49–55. <https://doi.org/10.3329/ralf.v9i1.59535>
- Selim, S., E. Hussein, & R. Abou-Elkhair. 2018. Effect of *Spirulina plantesis* as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. *Eur. Poult. Sci.* 82:1–13. <https://doi.org/10.1399/eps.2018.227>
- Sharoud, M. N. M. 2015. Protective effect of spirulina against paracetamol-induced hepatic injury in rats. *Journal Experimental Biology Agricultural* 3:44–53. <https://citeserx.ist.psu.edu/document?repid=rep1&type=pdf&doi=dc16e5d7009fd072ef7eab6a69d035f9cad76b>
- Sijabat, A. C. G., S. Isdadiyanto, & A. J. Sitaswi. 2023. Rat liver function induced by a high-fat diet after giving mahogany seeds ethanol extract. *J. Biol. Educ.* 15:230–236. <https://doi.org/10.15294/biosaintifika.v15i2.44632>
- Stadelman, W. J. & O. J. Cotterill. 1995. *Quality Identification of Shell Eggs*. In: *Egg Science and Technology*. Haworth Press, Inc., New York, NY. Pp. 39–66.
- Sugiharto, T. Yudiarti, I. Isroli, & E. Widiastuti. 2018. Effect

of feeding duration of *Spirulina plantesis* on growth performance, haematological parameters, intestinal microbial population and carcass traits of broiler chicks. S. Afr. J. Anim. Sci. 48:98–107. <https://doi.org/10.4314/sajas.v48i1.12>

- Sunarno, S., E. A. Kusuma, & A. J. Sitasiwi.** 2023. Protein and cholesterol levels of duck eggs after the addition of nanochitosan as a feed additive. J. Biol. Educ. 15:150-157. <https://doi.org/10.15294/biosaintifika.v15i2.41180>
- Suzana, D., F. D. Suyatna, R. Andrajati, S. P. Sari, & A. Mun'im.** 2017. Effect of *Moringa oleifera* leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia. J. Young Pharm. 9:79-84. <https://doi.org/10.5530/jyp.2017.1s.20>
- Tamzil, M. H. & B. Indarsih.** 2022. Thirty years development observation of Braekel chicken (*Gallus turnicus*) into Arabic chicken in Indonesia. Asian J. Anim. Sci. 16: 62-67. <https://doi.org/10.3923/ajas.2022.62.67>
- Tessier, R., J. Calvez, N. Khodorova, & C. Gaudichon.** 2021. Protein and amino acid digestibility of 15 N spirulina in rats. Eur. J. Nutr. 60:2263-2269. <https://doi.org/10.1007/s00394-020-02368-0>
- Tufarelli, V., P. Baghban-Kanani, S. Azimi-Youvalari, B. Hosseintabar-Ghasemabad, M. Slozhenkina, I. Gorlov, A. Seidavi, T. Ayasan, & V. Laudadio.** 2021. Effects of horsetail (*Equisetum arvense*) and spirulina (*Spirulina platensis*) dietary supplementation on laying hens productivity and oxidative status. Animals 11:335. <https://doi.org/10.3390/ani11020335>
- Vaz, B. S., J. B. Moreira, M. G. de Moraes, & J. A. V. Costa.** 2016. Microalgae as a new source of bioactive compounds in food supplements. Curr. Opin. Food Sci. 7:73–77. <https://doi.org/10.1016/j.cofs.2015.12.006>
- Zhou, Y., F. Tan, C. Li, W. Li, W. Liao, Q. Li, G. Qin, W. Lu, & X. Zhao.** 2019. White peony (fermented *Camellia sinensis*) polyphenols help prevent alcoholic liver injury via antioxidation. Antioxidants 8:524. <https://doi.org/10.3390/antiox8110524>
- Zeweil, H., I. M. Abaza, S. M. Zahran, M. H. Ahmed, H. M. Aboul-Ela, & A. S. Asmaa.** 2016. Effect of *Spirulina platensis* as dietary supplement on some biological traits for chickens under heat stress condition. Asian Journal Biomedical Pharmaceutical Sciences 6:8–12.