J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
48(1):47-57, March 2023
DOI: 10.14710/jitaa.48.1. 47-57

Dietary supplementation of *Spirulina platensis* and *Saccharomyces cerevisiae* on egg quality, physiological condition and ammonia emission of hens at the late laying period

H. I. Wahyuni*, T. Yudiarti, E. Widiastuti, T. A. Sartono, I. Agusetyaningsih, S. Sugiharto
Department of Animal Science, Faculty of Animal and Agricultural Sciences,
Universitas Diponegoro, Semarang, 50275, Indonesia
**Corresponding E-mail: hihannyiw123@gmail.com*

Received November 11, 2022; Accepted February 27, 2023

ABSTRACT

The study was aimed to evaluate the effect of *Spirulina platensis* and *Saccharomyces cerevisiae* or its combination on egg quality, physiological condition and ammonia excretion of hens at the late laying period. At 81 weeks old, 144 Lohmann Brown layer chickens were divided into four treatment groups included CON (hens fed basal feed), SP (basal feed + 0.3% *S. platensis*), SC (basal feed + 0.2% *S. cerevisiae*), and SPSC (basal feed + 0.3% *S. platensis* + 0.2% *S. cerevisiae*). At the end of the study, eggs, intestinal mucosa, digesta, excreta and blood sample were collected. Results showed that albumin index was higher (P<0.05) in SPSC group than in CON, SP and SC. The yolk index and yolk colour were greater (P<0.05) in SP and SPSC groups than in CON and SC. The erythrocyte values were higher (P<0.05) in SP group compared to CON, SC and SPSC groups. The ileum pH was higher (P<0.05) in SP than in CON, SC and SPSC. Lactic acid bacteria counts were lower (P<0.05) in the caecum of SC and SPSC than in CON and SP groups. The lower (P<0.05) counts of lactose-negative *Enterobacteriaceae* were shown in SC and SPSC than in CON. The excreta pH was lower (P<0.05) in SC group compared to CON, SP and SPSC groups. There were better (P<0.05) protein digestibility coefficient and nitrogen retention in SPSC group than others. Faecal ammonia decreased (P<0.05) in SP, SC and SPSC groups. In conclusion, *S. platensis* improved egg yolk index and colour, increased erythrocyte counts and played an important role in maintaining the balance of bacteria in the intestine resulting in reduced ammonia excretion. Dietary inclusion of *S. cerevisiae* reduced ammonia excretion of laying hens during the late laying period.

Keywords: Laying hens, Microalgae, Probiotic, Yeast

INTRODUCTION

Laying hens are one of the livestock commodities that primarily produce eggs. The rearing period of laying hens is typically divided into several phases, included starter, grower, laying and finisher phase. Laying hens start producing eggs in the pullet phase and decrease after reaching the peak of production. Molnar (2017) stated, laying hens are kept in production until they are

75-80 weeks old. Yet, farmers generally extend the egg production period till the age of 100 weeks, which is not only contributed to higher profitability but also more sustainable food production (Pottgüter, 2016). There were, however, a number of challenges, such as decreasing egg production and quality as well as the physiology of the hens in the later stages of commercial laying hens. It takes a diverse strategy to solve these problems.

Antibiotic growth promoters (AGP) were commonly used to maintain the productivity and health of laying hens during the late stage of egg production, but their use is now prohibited in the vast majority of countries worldwide included Indonesia (Wongsuvan *et al*., 2018; Afandi *et al*., 2020). *Spirulina platensis* has attracted the attention of poultry nutritionists because of its high protein content and other active ingredients (Mullenix *et al*., 2021). The microalgae *S. platensis* contains 53.31 g/100 g crude protein, 9.25 g/100 g lipids, 23.38 g/100 g carbohydrate, 7.44 mg/g chlorophyll a, 6.41 mg/g chlorophyll b, and 1.69 mg/g carotenoid (Rahim *et al*., 2021). *S. platensis* also contains antioxidant, immunomodulatory, anti-inflammatory, antiviral, and antimicrobial properties (Langers *et al*., 2012; Abdel-Daim *et al*., 2013; Shokri *et al*., 2014). In laying hens, dietary supplementation of *S. platensis* resulted in a significant improvement in egg physical characteristics (Omri *et al*., 2018; Zahroojian *et al*., 2013; Selim *et al*., 2018). Moreover, Mariey *et al*. (2012) showed that dietary inclusion of *S. platensis* (at 0.1, 0.15, and 0.2% of diets) increased egg weight, egg mass, and laying rate. In other commodities, the inclusion of 1% *S. platensis* into pig and broiler feeds was capable of improving the blood parameters, nutrient digestibility and performance, antioxidant activity, while reducing ammonia emission (Sugiharto *et al*, 2018; Park *et al*., 2018; Evans *et al*., 2015; Furbeyre *et al*., 2017).

Saccharomyces cerevisiae has been widely used as an additive and an alternative to AGP for poultry (Ogbuewu *et al*., 2018; Morales-Lopez and Brufau, 2013). *S. cerevisiae* contains vitamins, amino acids, and enzymes like mannases, glucanases, amylases, lipases, and proteases (Sugiharto, 2022; Ahiwe *et al*., 2021). The cell wall of *S. cerevisiae* is primarily composed of α-mannan (31% dry mass), mannoprotein, which accounts for approximately 40% dry mass, β-glucan (approximately 60% dry mass), and chitin (approximately 2% dry mass) (Ahiwe *et al*., 2021). Study in laying hens by Pinar (2013) and Swain *et al*. (2011) found that dietary supplementation of 1% or 1.5 g/kg *S. cerevisiae*, respectively, increased the percentage of hen day production, improved feed conversion ratio (FCR), increased albumen and shell thickness, increased egg weight, and improved feed efficiencies. Another study by Yalcin *et al*. (2014) found that adding yeast cell wall at 1 or 2 g/kg diets improved egg nutritional quality by lowering egg yolk cholesterol levels in 26-weeks brown laying hens.

It has been very common to combine several active ingredients to achieve synergistic and complementary effects on poultry. In this study, *S. platensis* and *S. cerevisiae* were used together for laying hens. Indeed, *S. platensis* contains a hepatotoxin called microcystin, which is toxic to the liver in high concentrations (Sugiharto *et al*., 2018). Study by Valerio *et al*. (2014) revealed that *S. cerevisiae* can ameliorate the toxic effect of microcystin in *S. platensis*. In this regard, the use of *S. platensis* and *S. cerevisiae* together was expected to get a synergistic effect from the two additives without causing toxic effects from *S. platensis*. So far, the use of combination of *S. platensis* and *S. cerevisiae* for commercial laying hens has not been studied. This study therefore aimed to evaluate the effect of *S. platensis* and *S. cerevisiae* or its combination on egg quality, physiological condition and ammonia excretion of hens at the late laying period

MATERIALS AND METHODS

Animals and Experimental Diets

One hundred forty four of 81 weeks old Isa Brown laying hens with an average body weight

Table 1. Nutritional Compositions of Basal Feeds

Items	Content (%)
Corn	55.0
Rice bran	18.0
Soybean meal	11.0
Corn gluten meal	5.00
Meat bone meal	3.00
Bone meal	2.00
Limestone	4.00
MCP	1.00
Premix**	1.00
Analysed nutritional composition*	
Metabolizable energy (kcal/kg)	2800
Crude protein	15.41
Crude fibre	7.00
Crude fat	3.00
Ash	12.00
Water content	13.00
Calcium	3.00
Phosphor	0.60

*Crude protein level was according to proximate analysis, while the other content corresponds to the feed label.
**Premix containing (per 10 kg of diet): Vitamin A 12,000,000 IU; Vitamin D3 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; Choline chloride 10,000 mg; Methionin 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200 mg; Copper 4,000 mg; Santoquine (antioxidant) 10,000 mg.

of 1,915±131.92 g from the commercial farm were used in this study. This study used an battery cage (20 × 40 cm^2). The temperature and humidity of the cage was between 22 to 30°C and 70 to 90%, respectively, while the light was provided intermittently every 2 hr from 7 PM until 5 AM. Four treatment groups with 6 replications for each group were arranged, so there were 24 experimental units. Each unit containing 6 laying hens that were divided into 3 cages, each with 2 hens. The treatment groups included CON (hens fed basal feed), SP (basal feed + 0.3% *S. platensis*), SC (basal feed + 0.2% *S. cerevisiae*), and SPSC (basal feed + 0.3% *S. platensis* + 0.2% *S. cerevisiae*). *S. platensis* was purchased from PT. Algaepark Indonesia Mandiri (Tangerang, Banten, Indonesia). Based on manufacturer's label, *S. platensis* contained 60-64 g/100 g protein, 5.6-7.2 g/100 g water, 20-24 g/100 g carbohydrates, <0.05 omega-6, 110-145 mg/100 g magnesium, 4.2-5.0 mg/100 4.2-5.0 mg/100 g vitamin B3, 1.5-2.5 mcg/100 mg vitamin B12, 6.0-7.2 mg/100 g vitamin E, 250-295 mg/1000 g carotene, 720-840 mg/100 g chlorophyll, 9150-10450 mg/100 g phycocyanobilin. *S. cerevisiae* contained 9.82 × 10^{11} cfu/g. The yeast was purchased from Angel Yeast Co. Ltd., Hubei, China.

The hens were provided with manual feeders and drinking water facilities. The nutrition content of basal feed was 13% water, 16% protein, 3% fat, 7% crude fibre, 12% ash, 3% calcium, and 0.6% phosphor, with 2800 kcal/kg metabolizable energy. From the beginning to the end of the rearing period, drinking water was provided *ad libitum*, while the feed was offered twice a day (morning and afternoon). The treatment lasted 35 days. Egg was taken from each experimental unit in the last 3 days of the study (33-35 days) to determine the egg quality. Following that, blood and excreta samples were collected at the last day of the experiment.

Data Collection and Laboratory Analysis

Egg Quality

Observation on egg quality were carried out at the end of the study. One egg was collected for each experimental unit to be examined for albumin index, yolk index, yolk colour, Haugh unit, shell thickness and egg weight. The height of albumen and yolk was measured using a standard micrometre, while yolk diameter was determined using calliper. Shells were washed using water and dried, then shell thickness was measured by micrometre. Haugh units was calculated by formula as described by Khaleel *et al*. (2019) as follows = $100*\log(H+7.57)-(1.7*WE^{0.37})$. Yolk index was determined by dividing the yolk height by yolk diameter.

Complete Blood Counts

Blood sample was collected from the wing vein of each hen for complete blood count determination. The collected blood was placed in ethylenediaminetetraacetic acid (EDTA)-containing vacutainers prior to analysis. Routine haematology measurements were carried out with a fully automatic haematology analyser from PT. Prima Alkesindo Nusantara (PRIMA, Power 100-400V~50/60Hz 150 VA).

Intestinal pH

Twenty-four laying hens were used to assess the intestinal pH from intestine. The digestive tract was immediately prepared after euthanasia. A pH meter (OHAUS ST300) was used to measure the pH of the duodenum, jejunum, ileum, and caecum.

Ileum and Caecum Bacterial Population

The determination of intestinal bacterial population of the ileum and caecum was conducted according to Sugiharto *et al*. (2022). After euthanasia, the digesta of ileum and caecum were transferred into 15 mL containers and immediately analysed for the number of lactic acid bacteria (LAB), coliform and lactose-negative bacteria (LNE). Viable counts of LAB in the digesta sample were conducted by plating onto De Man Rogosa and Sharpe (MRS) agar and on MacConkey agar plates for coliform and LNE. The incubation of the MRS agar medium was carried out anaerobically for 48 h at 37°C and 24 hours at 37°C under aerobic condition for MacConkey agar. After incubation, the bacterial colonies were counted and presented in log cfu/g.

Water Content, Acidity, Temperature of Excreta and Protein Digestibility

Sample of excreta were collected at the end of study. Total nitrogen (N) was determined by standard Kjehdahl method. The Kjehdahl procedure was adopted for the crude protein measurement (Jabbar *et al*., 2020). Measurement of ammonia level was carried out using ammonia detector (Smart Sensor AR8500, Intell Instrumens Plus) with accuracy of 1 ppm according to the manufacturer instruction. Excreta temperature was determined by inserting a thermometer (ThermoONE, Onemed) into hen excreta. Moisture content was measured by weighing the wet weight and dry weight of excreta. Nitrogen retention was calculated according to Tillman (1998). Nitrogen retention was measured by calculating the amount of nitrogen consumed minus the nitrogen excretion corrected for endogenous nitrogen. The formula for calculating the nitrogen retention is as follow = N consumption – (N Excreta – N Endogenous)

Statistical Analysis

SPSS program version 16.0 (IBM SPSS Statistics for Windows) was used to analyse data. The data obtained were statistically analysed using one-way ANOVA with a 5% significance level. Duncan's multiple range test was used to evaluate the variations among treatment groups.

RESULTS

Egg Quality

Table 2 shows data on egg quality. The treatment of SP, SC, and SPSC had substantial effects ($P<0.05$) on albumin index, yolk index, and yolk colour. The albumin index was higher in the SPSC group than in the CON, SP, and SC

groups. There was no significant difference in albumin index between CON, SP, and SC groups. In contrast to the previous parameters, the SP and SPSC groups had differences (P<0.05) in yolk index and yolk colour when compared to the CON group, but the SC group was not significantly different when compared to the CON or SP and SPSC groups. The treatment had no effect (P>0.05) on Haugh Unit, shell thickness, or egg weight.

Complete Blood Counts

Data on complete blood counts of laying hens are listed in Table 3. The values of erythrocytes were greater (P<0.05) in SP group than in CON, SC and SPSC. There was no influence (P>0.05) of treatments on the serum concentrations of haemoglobin, haematocrits, MCV, MCH, MCHC, leukocytes, heterophils, lymphocytes and thrombocytes in laying hens.

Small Intestine pH

Data on the small intestine pH of laying hens are listed in Table 4. The pH level of ileum was higher (P<0.05) in SP than in CON, SC and SPSC groups. There was no significant change in pH in all groups.

Intestinal Ecology of Laying Hens

The bacterial populations in the intestine are presented in Table 4. There was no difference (P>0.05) in the numbers of LAB, coliform and LNE enumerated in ileum. However, the SP and SPSC groups showed a decrease (P<0.05) in LAB populations when compared to CON and SC groups. Likewise, the LNE population in the

Table 2. Egg Quality of Laying Hens

Items	CON	SP	SC	SPSC	SEM	P value
Albumin index	0.083[b]	0.095[ab]	0.093[ab]	0.108[a]	0.032	0.046
Yolk index	0.308[b]	0.331[a]	0.330[ab]	0.332[a]	0.003	0.012
Yolk colour	11.77[b]	12.27[a]	11.77[ab]	12.05[a]	0.147	0.019
Haugh Unit	78.73	82.61	84.18	86.83	0.006	0.113
Shell thickness (mm)	0.52	0.49	0.48	0.48	0.007	0.195
Egg weight	65.00	64.14	64.18	64.43	0.234	0.563

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05)

CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean

Table 3. Complete Blood Counts of Layer Chickens

Items	CON	SP	SC	SPSC	SEM	P value
Haemoglobin (g/dL)	10.67	12.33	10.25	10.83	0.12	0.349
Erythrocytes (10^6/µL)	3.00[b]	3.66[a]	2.77[b]	3.01[b]	0.43	0.038
Haematocrit (%)	38.67	44.67	36.08	38.67	1.52	0.238
MCV (fL)	128.81	122.18	130.45	128.36	1.63	0.312
MCH (pg)	35.52	35.25	37.00	35.88	1.52	0.238
MCHC (g/dL)	27.62	27.58	28.42	28.02	0.25	0.624
Leukocytes (10^3/µL)	77.50	80.25	73.16	77.00	2.81	0.865
Heterophils (10^3/µL)	3.00	2.08	2.75	1.83	0.20	0.112
Lymphocytes (10^3/µL)	74.50	78.16	70.41	75.16	2.78	0.825
Thrombocytes (10^3/µL)	9.16	11.50	10.66	9.00	0.58	0.375

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05)

CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

SC and SPSC groups decreased (P<0.05) compared to CON. Laying hens treated with SP did not show a significant difference from the CON, SC and SPSC groups.

Water Content, Acidity and Excreta Temperature

Data of water content, acidity and excreta temperature of laying hens are listed in Table 6. At the end of study, the treatments applied had no significant effect (P>0.05) on water content (%) and excreta temperature. In the contrary, pH of excreta was significantly affected by the treatment. The hens in SC group have a greater excreta pH than others (CON, SP and SPSC).

Protein Digestibility

Research data on protein digestibility are shown in Table 7. It was shown that the treatment had an effect on the protein digestibility coefficient, nitrogen retention and excreta ammonia. The SC group had a lower protein digestibility coefficient (P<0.05) compared to SPSC group. Higher N retention (P<0.05) found in SPSC group than in SC. Treatment with SP, SC and SPSC lowered (P<0.05) the ammonia content in laying hen excreta. However, the treatment had no significant effect on N excreta.

DISCUSSION

S. platensis and *S. cerevisiae* have received attention as a potential alternative to AGP that can maintain production performance, and also acts as health promoting agent in poultry. Although having no positive influence on the egg production, feed consumption, FCR and mortality (unpublished data), data in the present study showed that the albumin index increased in the SPSC group. Furthermore, the yolk index and yolk colour in the SP and SPSC groups increased significantly when compared with the CON and SC groups. Indeed, these present findings were consistent with those of Dogan *et al*. (2016), who revealed that feeding 2% *S. platensis* increased quail yolk index. Different from our study, Selim

Table 4. Small Intestine pH of Laying Hens

Items	CON	SP	SC	SPSC	SEM	P value
Duodenum	6.27	6.38	6.58	6.04	0.07	0.081
Jejunum	6.30	6.55	6.37	6.07	0.09	0.305
Ileum	6.58[b]	7.34[a]	6.90[b]	6.60[b]	0.08	0.001
Cecum	7.00	7.37	7.29	7.05	0.08	0.284

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05)
CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean

Table 5. Bacterial Populations in Ileum and Caecum of Laying Hens

Items	CON	SP	SC	SPSC	SEM	P value
Ileum (log CFU/g)						
LAB	11.51	11.51	11.44	11.36	0.074	0.900
Coliform	6.03	5.70	5.75	5.55	0.208	0.891
Lactose negative-enterobacteria	7.48	7.82	6.85	5.55	0.367	0.127
Caecum (log CFU/g)						
LAB	11.70[a]	11.64[a]	11.19[b]	11.17[b]	0.082	0.021
Coliform	6.96	7.33	6.34	6.48	0.287	0.629
Lactose negative-enterobacteria (LNE)	9.08[a]	7.74[ab]	6.90[b]	6.80[b]	0.333	0.045

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05)
CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean

Table 6. Water Content, Acidity and Temperature of Laying Hens Excreta

Items	CON	SP	SC	SPSC	SEM	P value
Water content (%)	84.7	79.9	81.7	84.6	0.004	0.274
pH	6.7[b]	6.5[b]	6.8[a]	6.7[b]	0.029	<0.001
Temperature (°C)	33.8	34.2	34.5	34.3	0.133	0.271

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05)
CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean

Table 7. Protein Digestibility of Laying Hens

Items	CON	SP	SC	SPSC	SEM	P value
Protein digestibility coefficient (%)	57.76[ab]	60.96[ab]	48.60[b]	70.40[a]	2.73	0.031
Nitrogen retention	3.80[bc]	4.07[ab]	3.01[c]	4.90[a]	0.21	0.009
Nitrogen excreta	5.29	5.26	5.65	4.59	0.16	0.124
Faecal ammonia	14.2[a]	7.0[b]	6.8[b]	6.7[b]	0.818	<0.001

[a,b,c]Means marked with superscript letters in the same row are significantly different (P<0.05)
CON (hens fed by basal feed), SP (basal feed + *S. platensis* 0.3%), SC (basal feed + S. cerevisiae 0.2%), and SPSC (basal feed + S. platensis 0.3% + *S. cerevisiae* 0.2%), SEM: standard error of the mean

et al. (2018) found no significant effect of *S. platensis* on albumin index and yolk index of hens. The higher flavonoid content of *S. platensis* added to feed can explain the improvement in yolk colour in the SP and SPSC groups (Tufarelli *et al*., 2021). The latter authors further revealed that higher flavonoid levels in feed corresponded to higher pigment deposition in yolk, which determined colour intensity. Moreover, the effect of *S. platensis* on yolk colour may also be due to the biomass's β-carotene content due to *S. platensis* treatment (Khan *et al*., 2021).

It was apparent in this present study that, treatment with *S. platensis* increased erythrocyte counts in laying hens. Similar to this study, Sarker *et al*. (2022) reported the increased concentration of erythrocytes in laying hen blood after supplementation with *S. platensis*. In this case, erythrocytes improved due to phycocyanine, a bioactive compound in *S. platensis* that can stimulate erythropoietin hormone production (Sarker *et al*., 2022). This hormone stimulates stem cells in the bone marrow to produce more red blood cells. Similar to Sarker *et al*. (2022), Pankaj and Varma (2013) discovered that oral administration of *S. platensis* to rats increased red blood cell count.

In the current study, feeding *S. cerevisiae* and combining it with *S. platensis* reduced the population of LAB and LNE in the caecum digesta of laying hens. With regard particularly to *S. cerevisiae*, such probiotic yeast has reportedly been shown to possess antimicrobial characteristics, hence lowering the number of dangerous bacteria, such as LNE, in the intestine of poultry (Sugiharto *et al*., 2022). Different from Erya *et al*. (2020) showing no effect of *S. cerevisiae* on the caecal LAB population, results in this present study showed reduced caecal LAB counts due to *S.* cerevisiae treatment. So far, the exact reason for such condition remains unknown. In term of *S. platensis*, flavonoid compound of *S. platensis* was actually expected to function as a bacterial clearing agent (Akbarbaglu *et al*., 2022). In accordance with this, *S. platensis* administration resulted in reduced caecal LNE content in this study, although the values did not reach the significant levels. In line with our findings, Nuhu (2013) revealed that *S. platensis* was effective at inhibiting the growth of harmful bacteria in the intestine as a result of antibacterial activity.

The water content of laying hen excreta ranged from 79.9 to 84.7% in this study. The treatment had no discernible effect on the excreta water content in any of the treatment groups

because the basal feed distributed to each group had the same nutritional composition. Indeed, high protein encourages high water content in excreta because of higher glomerulus uric acid concentration, which causes laying hens to drink more water. Due to the little percentage inclusion in this investigation, it seemed as *S. platensis* inclusion did not significantly increase the crude protein content of the basal diets. The excreta temperatures in the SP, SC, and SPSC groups did not differ significantly from one another. This suggests that the amount of metabolizable energy in each group was equal to the amount required to maintain a normal body temperature, indicating that there were no appreciable differences in homeostasis between the treatment groups.

In this study, *S. platensis*, *S. cerevisiae*, and both combinations were found to reduce ammonia levels in excreta. The dietary supplementation of *S. platensis* and *S. cerevisiae* was thought to have resulted in optimal intestinal microbe function. The probiotic microbes could inhibit the activity of the urease enzyme (Rezaee *et al*., 2019), which can reduce the amount of uric acid in the digestive tract of laying hens. Acid producing bacteria combined with oligosaccharide-containing ingredients were more effective at reducing ammonia. The level of ammonia in excreta is typically determined by the amount of N in the excreta (Jeong and Kim, 2014). However, there was no significant effect of N excreta treatment in this study. The higher protein digestibility coefficient in the SPSC group compared to the SC group suggests that both supplements had a synergistic effect. Protein digestibility appears to be related to the balance of essential and non-essential amino acids in *S. platensis*, which can aid in the digestion of crude protein in poultry (Park *et al*., 2018; Bleakley and Hayes, 2017; Evans *et al*., 2015).

Maximum protein digestibility can increase N retention, as demonstrated by the findings of this study. Nitrogen retention is the amount of nitrogen that hens absorb and use. The results showed that higher N retention in SPSC groups corresponded to lower excreta ammonia in the SPSC group. This means that N from amino acids was used more for production and maintaining health than nitrogen from the consumed ration (Park *et al*., 2018).

Overall, several parameters showed better results with single administration of *S. platensis* or *S. cerevisiae*, when compared to the combined administration of *S. platensis* and *S. cerevisiae*. This may be because the combination of *S. platensis* and *S. cerevisiae* does not always have a synergistic effect on poultry conditions (Sugiharto *et al*., 2022). The results of this study indicate that single administration of *S. platensis* or *S. cerevisiae* will be more efficient in improving egg quality and health of laying hens in the period after peak production.

In conclusion, *S. platensis* improved yolk index and colour, increased erythrocyte counts and played an important role in maintaining the balance of microbes in the digestive tracts resulting in reduced ammonia excretion. Dietary inclusion of *S. cerevisiae* reduced ammonia excretion of laying hens during the late laying period.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENTS

Author wishing to acknowledge the research students, laboratory technicians and financial support from Universitas Diponegoro.

REFERENCES

Abdel-Daim, M.M., S.M. Abuzead and S.M. Halawa. 2013. Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats. PLoS One. 8(9):e72991.

Afandi, R., B. Hartono and I. Djunaidi. 2020. The analysis of production costs of laying

hen farms using semi self-mixing and total self-mixing feeds in Blitar Regency, East Java. Trop. Anim. Sci. J. 3(1):70-76.

Ahiwe, E.U., T.T. Tedeschi Dos Santos, H. Graham and P.A. Iji. 2021. Can probiotic or prebiotic yeast (*Saccharomyces cerevisiae*) serve as alternatives to in-feed antibiotics for healthy or disease-challenged broiler chickens?: a review. J. Appl. Poult. Res. 30 (3):100164.

Akbarbaglu, Z., A. Ayaseh, B. Ghanbarzadeh and K. Sarabandi. 2022. Techno-functional, biological and structural properties of *Spirulina platensis* peptides from different proteases. Algal Res. 66: 102755.

Bleakley, S. and M. Hayes. 2017. Algal proteins: extraction, application, and challenges concerning production. Foods. 6(5):33.

Dogan, S.C., E. Hussein and R. Abou Akbar. 2016. Effect of *Spirulina platensis* as feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. Eur. Poult. Sci. 82:1-14.

Erya, S.N., H.I. Wahyuni, T. Yudiarti, E. Widiastuti, and S. Sugiharto. 2020. Intestinal morphology and growth performance of the Indonesian indigenous crossbred chickens supplemented with formic acid and *Saccharomyces cerevisiae*. J. Indonesian Trop. Anim. Agric. 45(4):348-355.

Evans, A.M., D.L. Smith and J.S. Moritz. 2015. Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance. J. Appl. Poult. Res. 24(2):206-214

Furbeyre, H., J. van Milgen, T. Mener, M. Gloaguen and E. Labussiere. 2017. Effects of dietary supplementation with fresh water microalgae on growth performance, nutrient digestibility, and gut health in weaned piglets. Animal. 11(2):183-192

Jabbar, A., M. Tahir, R.U. Khan, and N. Ahmad. 2020. Interactive effect of exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase. Trop. Anim. Health Prod. 53(1):23.

Jeong, J.S. and I.H. Kim. 2014. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult. Sci. 93(12):3097-3103.

Khaleel, R.M.T. 2019. Prediction of Haugh Unit through albumen height and egg weight. Mesopotamia J. Agric. 47:37-43.

Khan, F., A.A.S. Shuvo and K.M.S. Islam. 2021. Effect of dietary inclusion of *Spirulina platensis* on egg yolk pigmentation. Livest. Res. Rural Dev. 33(8):101.

Langers, I., V.M. Renoux, M. Thiry, P. Delvenne and N. Jacobs. 2012. Natural killer cells: role in local tumor growth and metastasis. Biologics. 6:73-82.

Mariey, Y.A., H.R. Samak and M.A. Ibrahem. 2012. Effect of using *Spirulina platensis* algae as a feed additive for poultry diets. 1. Productive and reproductive performance of local laying hens. Egypt. Poult. Sci. 32 (1):201-215.

Molnar, A. 2017. Extended production cycle in laying hens-the role of nutrition and management. Ph.D. thesis KU Leuven, Belgium. DOI: 10.13140/RG.2.2.20930.27841

Morales-Lopez, R. and J. Brufau. 2013. Immunemodulatory effects of dietary Saccharomyces cerevisiae cell wall in broiler chickens inoculated with *Escherichia coli* lipopolysaccharide. Br. Poult. Sci. 54 (2):247-251.

Mullenix, G.J., C.J. Maynard, C.M. Owens, S.J. Rochell, W.G. Bottje, R.D. Brister and M.T. Kidd. 2021. *Spirulina platensis* meal inclusion effects on broilers fed a reduced protein diet. J. Appl. Poult. Res. 31(1):100199.

Nuhu, A.A. 2013. *Spirulina* (*Arthrospira*): An important source of nutritional and medical compounds. J. Mar. Biol. DOI: http://dx.doi.org/10.1155/2013/325636

Ogbuewu, I.P., V.M. Okoro, E.F. Mbajiorgu and C.A. Mbajiorgu. 2019. Yeast

(*Saccharomyces cerevisiae*) and its effect on production indices of livestock and poultry - a review. Comp. Clin. Pathol. 28: 669-677.

Omri, B., M. Amraoui, A. Tarek, M. Lucarini, A. Durazzo, N. Cicero, A. Santini and M. Kamoun. 2019. *Arthrospira platensis* (*Spirulina*) supplementation on laying hens' performance: egg physical, chemical and sensorial qualities. Foods. 8(9):386.

Pankaj, P.P. and M.C. Varma. 2013. Potential role of Spirulina platensis in maintaining blood parameters in alloxan induced diabetic mice. Int. J. Pharm. Pharm. Sci. 5(4): 450 -456.

Park, J.H., S.I. Lee and 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(7):2451-2459.

Pinar, S., E. Ahmed, H.K. Bekir, O. Bulent and C. Zafer. 2011. Effect of inactivated brewer's yeast (*Saccharomyces cerevisiae*) on egg production, serum antibody titres and cholesterol levels in laying hens. Vet. ir Zootech. 61(83):53-60.

Pottgüter, R. 2016. Feeding Laying Hens to 100 Weeks of Age. LOHMANN Information. 50(1):18-21.

Rahim, A., C. Cakir, M. Ozturk, B. Sahin, A. Soulaimani, M. Sibaoueih, B. Nasser, R. Eddoha and A. Assamadi. 2021. Chemimcal characterization and nutritional value of *Spirulina platensis* cultivated in natural conditions of Chichaoua region (Morocco). S. Afr. J. Bot. 141:235-242.

Rezaee, P., R.K. Kermanshahi and T. Falsafi. 2019. Antibacterial activity of lactobacilli probiotics on clinical strains of *Helicobacter pylori*. Iran J. Basic Med Sci. 22 (10):1118-1124.

Sarker, M.S., K. Rafiq, M.M. Rahman, K.K.I. Khalil, S. Islam and M.S. Islam. 2022. Effect of *Spirulina* (*Spirulina platensis*) on production, hematological parameters and lipid profile in layers. Res. Agric. Livest. Fish. 9(1):49-55.

Selim, S., E. Hussein and R. Abou-Elkhair. 2018. Effect of *Spirulina platensis* as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. Europ. Poult. Sci. 82:1-13.

Shokri, H., A. Khosravi and M. Taghavi. 2014. Efficacy of *Spirulina platensis* on immune functions in cancer mice with systemic candidiasis. J. Mycol. Res. 1(1):7-13

Sugiharto, S., B.M. Atmaja, E. Widiastuti and H. Hadiyanto. 2022. Combined use of *Spirulina platensis* and *Saccharomyces cerevisiae*: Implication on growth, blood profile, and intestinal morphology and bacteria of the Indonesian crossbred chickens. Biodiversitas. 23(1):160-165.

Sugiharto, S., T. Yudiarti, I. Isroli and E. Widiastuti. 2018. Effect of feeding duration of *Spirulina* platensis on growth performance, haematological parameters, intestinal microbial population and carcass traits of broiler chicks. S. Afr. J. Anim. Sci. 48(1):98-107.

Swain, B.K., P.K. Naik, E.B. Chakurkar and N.P. Singh. 2011. Effect of probiotics and yeast supplementation on performance, egg quality characteristics and economics of production in Vanaraja layers. Indian Poult. Sci. 46:313-315.

Tillman, A.D. 1998. *Basic Animal Feed Sciences*. Yogyakarta. Gadjah Mada University Press.

Tufarelli, V., P. Baghban-Kanani, S. Azimi-Youvalari, B. Hosseintabar-Ghasemabad, M. Slozhenkina, I. Gorlov, A. Seidavi, T. Ayasan, and V. Laudadio. 2021. Effect of Horsetail (*Equisetum arvense*) and *Spirulina* (*Spirulina platensis*) dietary supplementation on laying hens productivity and oxidative status. Animals, 11(2):335.

Valerio, E., A. Vilares, A. Campos, P. Pereira and V. Vasconcelos. 2014. Effects of microcystin-LR on *Saccharomyces cerevisiae* growth, oxidative stress and apoptosis. Toxicon. 90:191-198.

Wongsuvan, G., V. Wuthiekanun, S. Hinjoy, N.P. Day and D. Limmathurotsakul. 2018. Antibiotic use in poultry: A survey of eight farms in Thailand. Bull. World Health Organ. 96(2):94-100.

Yalcin, S., I. Onbasilar, E.H. Handan and A. Sahin. 2014. Effect of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens. Ankara Univ. Vet. Fak. Derg. 61(4):289-294.

Zahroojian, N., H. Moravej and M. Shivazad. 2013. Effects of dietary marine algae (*Spirulina platensis*) on egg quality and production performance of laying hens. J. Agr. Sci. Tech, 15(7):1353-1360.