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Combined use of *Spirulina platensis* and *Saccharomyces cerevisiae*: Implication on growth, blood profile and intestinal morphology and bacteria of the Indonesian crossbred chickens

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Abstract. *Sugiharto S, Atmaja BM, Widiastuti E, Hadiyanto H. 2021. 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: 160-165.* The effects of *Spirulina platensis* and *Saccharomyces cerevisiae* on poultry growth and health have long been known. However, no research has been conducted on the combined use of these substances on the Indonesian crossbred chicken (ICC). This study evaluated how the combined use of *S. platensis* and *S. cerevisiae* affected ICC growth, blood profile, and intestinal ecology. A total of 288 ICC were separated into three groups, including CONT (basic diet), SP (diet with 0.3% *S. platensis*), and SPSC (diet with 0.3% *S. platensis* and 0.2% *S. cerevisiae*). Blood and intestinal content and segments were taken at week 9, while growth was determined weekly. SP had greater ($p<0.05$) body weight than CONT, but did not differ from SPSC. In SP, ileal coliform counts were lower ($p=0.07$) than in CONT. SPSC had lower ($p=0.07$) caecal *Enterobacteriaceae* counts than other groups. Blood profile, intestinal morphology, organ weight, and carcass traits did not vary ($p>0.05$) across the ICC. In conclusion, dietary supplementation of *S. platensis* improved growth performance and bacterial population of the ICC.

Keywords: Crossbred chicken, health, microalgae, probiotics yeast, production variable

INTRODUCTION

The Indonesian crossbred chicken (ICC), which is a cross between a male Indonesian native chicken and a female layer, has lately attracted more interest from the Indonesian chicken meat customers. The meat of the crossbred chicken is tastier and more delectable than that of modern broiler chickens (Chen et al. 2016). As a result of the recent rise in the local market, farmers are now encouraged to enhance the productivity of the ICC. With the intensive farming system, the ICC can be harvested during week 8 to 10 with 800 to 900 g/bird (Sugiharto et al. 2019; 2021). Antibiotic growth promoters (AGP) were widely used in the diets of ICC to enhance their growth and maintain health conditions. However, from January 1, 2018, the use of AGP in chicken diets has been banned in Indonesia owing to consumer health concerns (the possibility of human antibiotic resistance). In practice, removing AGP from diets had a negative impact on chicken production output and raised death rates (Sugiharto 2016). As a result, alternative AGP replacements are critical for the ICC's long-term production.

For a long time, algae has been considered a potential source of protein for chickens. Among the algae, *Spirulina* (*Arthrospira*) *platensis* contains high protein (55-70%) and amino acid that resembles soybean meal, and may therefore be a viable alternative protein source for chickens (Mullenix et al. 2021a,b). In this case, *S. platensis* has been included in diets as a source of protein and exerted growth promoting effect on broiler chickens (Islam et al. 2021; Moustafa et al. 2021). *Spirulina platensis* also contains a variety of functional components such as vitamins, essential fatty acids, minerals, antioxidants and pigments, which are beneficial for the health conditions of chickens (Liestianty et al. 2019; Islam et al. 2021). Indeed, the use of *S. platensis* has been found to modulate the immune responses (Mullenix et al. 2021b), balance the antioxidant (redox) status (Moustafa et al. 2021) and improve physiological conditions (Islam et al. 2021) of broiler chickens. Moreover, *S. platensis* contains oligosaccharide that are essential for the growth of lactic acid bacteria (*Lactobacillus paracasei* and *Bifidobacterium animalis*) as reported by Cai et al. (2022). Likewise, the application of *S. platensis* has been demonstrated to improve intestinal morphology (increased villi height and goblet cell numbers) and hence growth performance of broilers (Khan et al. 2020).

Saccharomyces cerevisiae is a yeast-based probiotic that has long been used to protect poultry against diseases while also boosting their growth (Sugiharto 2016). *Saccharomyces cerevisiae* was recently added to the ICC's diets, and it was discovered that the yeast increased the chickens' growth, feed efficiency, and economic performance (Sugiharto et al. 2019). According to the literature (Sapsuha et al. 2021), probiotics may be coupled with other active compounds to optimize the probiotic's impact on poultry. In the study by Sugiharto et al. (2019), *S. cerevisiae* was mixed with formic acid and offered to the ICC. They discovered that combining *S. cerevisiae* and formic acid improved the ICC's production and economic performance more effectively than using either *S. cerevisiae* or formic acid separately. In light of the foregoing, *S. cerevisiae* was coupled with *S. platensis* in this work to get a synergistic impact of *S. cerevisiae* and *S. platensis* on the ICC's production performance and health. To our knowledge, the combined usage of *S. platensis* and *S. cerevisiae* in poultry, including the ICC, has never been published. Therefore, the present study aimed to investigate the effect of combined use of *S. platensis* and *S. cerevisiae* on growth, blood profile and intestinal morphology and bacteria of the ICC.

MATERIALS AND METHODS

The broiler chicken study was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang Indonesia (No. 57-07/A3/KEP/FPP). A total of 288 unsexed ICC were fed formulated starter (week 0-4) and finisher (week 4-9) feeds (Table 1). The chicks were divided into three groups upon arrival, including CONT (chicks provided with basal diet), SP (diet supplemented with 0.3% *S. platensis*), and SPSC (diet supplemented with 0.3% *S. platensis* and 0.2% *S. cerevisiae*). *Spirulina platensis* meal was obtained from PT. Algaepark Indonesia Mandiri (South Tangerang, Banten, Indonesia) containing of 60-64% protein, 5.6-7.2% moisture, 20-24% carbohydrate, <0.05% omega-6, 110 - 145 mg/100 g magnesium, 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 β-carotene, 720 - 840 mg/100 g chlorophyll, 9150 - 10450 mg/100 g phycocyanobilin (based on the manufacturer label). According to the proximate analysis, *S. platensis* contained 91.8% moisture, 52.4% crude protein, 0.63% crude fat, 34.2% crude fiber and 11.9% ash (based on dry matter). The *S. cerevisiae* was obtained from Angel Yeast Co. Ltd., Hubei, China, containing of 9.82×10^{11} cfu/g. With three treatment groups and six replicates/pens (16 chicks in each pen), the experiment was set up using a fully randomized design (CRD). The chicks were raised in an opened-broiler house with a 1.5 × 1.5 m pen. The broiler house's temperature and relative humidity were controlled by light bulbs and blower. Rice husk bedding was used to raise the chicks. Throughout the experiment, the continuous (24 hours) light program was applied. Newcastle disease vaccine was given to the chicks on days 4 (by eye drop) and 20 (through drinking water). Gumboro vaccine was also given by drinking water on day 11. The body weight (individually weighed) of the chicks, their feed intake, and their feed conversion ratio (FCR) were all monitored on a weekly basis. The difference between the amount of feed provided and the leftover feed was used to determine feed intake, while the FCR was obtained by dividing feed intake by broiler weight gain.

At week 9 (the period when the ICC's body weight was expected to reach the marketing weight), blood was collected from the wing vein of two chicks per pen (12 chicks per treatment group). For full blood counts, the blood was placed in a vacutainer containing ethylenediaminetetraacetic acid (EDTA). According to the manufacturer's description, the Prima Fully-Auto Hematology Analyzer (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) was used to measure the full blood counts. The bird was slaughtered after the blood was withdrawn from the wing's vein. Internal organs were subsequently taken from the birds and weighed (in empty condition). The digesta was collected from the duodenum, jejunum, and ileum for pH measurement (using the Portable pH Meter OHAUS ST300). The digesta from the ileum and caecum of broilers was also collected and placed in sterile bottle for the enumeration of bacterial population. The duodenum, jejunum, and ileum segments (about 2 cm for each) were collected for the intestinal histological evaluation. These intestinal segments were then submerged in a 10% neutral formalin buffer until analysis. In addition, the carcass and commercial parts of the birds (breast, wings, thigh, drumstick, and back) were examined.

After 24 hours of aerobic incubation at 38°C, coliform and lactose-negative *Enterobacteriaceae* were counted as red and colorless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). *Enterobacteriaceae* was defined as the sum of coliform and lactose-negative *Enterobacteriaceae*.

Table 1. Feedstuffs and nutritional compositions of feeds

Variables (%, unless otherwise noted)	Starter	Finisher
Yellow maize	53.5	61.0
Palm oil	2.32	2.95
SBM	40.1	32.0
DL-methionine	0.19	0.19
Bentonite	0.75	0.75
Limestone	1.00	1.00
MCP	1.30	1.30
Premix	0.35	0.35
Chlorine chloride	0.09	0.06
Salt	0.40	0.40
Calculated nutritional compositions:		
ME, (kcal/kg)[1]	2,900	3,023
Crude protein	22.0	19.0
Crude fiber	5.47	5.53
Analyzed nutritional compositions		
Moisture	11.6	10.3
Crude protein	21.9	20.5
Crude fiber	9.06	8.79
Crude fat	6.62	4.97
Ash	9.48	11.1

Note: [1]ME (metabolizable energy) was estimated based on formula (Bolton 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}; SBM: soybean meal, MCP: monocalcium phosphate

The quantities of lactic acid bacteria (LAB) were counted after anaerobic incubation at 38°C for 48 hours on de Man Rogosa and Sharpe (MRS) agar (Merck KGaA). The histological measurement of the small intestinal segment of broilers was conducted according to the procedure described by Tunç et al. (2019). Staining of 5 µm intestinal slices was done using hematoxylin and eosin. Using an optical microscope connected to a digital camera, the villi heights and crypt depths were measured. The average villi height and crypt depth were calculated using five measurements for each bird.

The data were statistically handled using analysis of variance in accordance with CRD (ANOVA, SPSS 16.0 version). When dietary interventions had a significant effect ($p<0.05$), the Duncan multi-range test was used. Tendency was considered when $0.05<p\leq0.10$.

RESULTS AND DISCUSSION

Data on performance of ICC are outlined on Table 2. On week 4, FCR was lower ($p<0.05$) in SP than that in CONT and SPSC groups. Final body weight and body weight gain of ICC tended ($p=0.06$) to be higher in SP and SPSC than in CONT. The SPSC birds tended ($p=0.07$) to consume more feed than other birds on week 4. On week 9, final body weight and body weight gain were higher ($p<0.05$) in SP than that in CONT, but did not differ from that of SPSC group. The treatments had no significant effects on feed intake and FCR on week 9. In general, the ICC's final body weight in this study was consistent with Sugiharto et al. (2021), who observed that the ICC's body weight at 10 weeks of age was roughly 868 g. In this study, dietary supplementation with *S. platensis* resulted in an increase in ICC's final body weight and body weight gain at week 9. This finding corroborated Khan et al. (2020), in which supplementing broilers with *S. platensis* increased their growth performance. In agreement, Kaoud (2012) documented that dietary administration of *S. platensis* increased daily weight gain as well as live body weight of broilers at day 42. In this regard, some researchers have hypothesized that *S. platensis* contains high-quality protein as well as growth-promoting substances that could help ICC grow faster (Islam et al. 2021; Moustafa et al. 2021). With respect particularly to *S. cerevisiae*, Kaoud (2012) previously demonstrated that feeding mannose-oligosaccharides (MOS) derived from the cell wall of *S. cerevisiae* resulted in greater live body weight of broilers at day 42 when compared to control. According to the latter author, the prebiotic effect of MOS (from *S. cerevisiae*) was responsible for improved intestinal microbial balance and digestion, and hence improved chicken growth performance. Contrary to our expectations, the combination of *S. platensis* and *S. cerevisiae* did not boost growth rate, as the growth performance of chicks did not substantially differ between SPSC and CONT group. The cause of the latter circumstance was unknown, but it was assumed that *S. cerevisiae* used some amino acids with growth-promoting properties, such as glutamine, as a nitrogen source (Chen and Kaiser 2002). As a result, the growth-promoting impact of *S. platensis* on ICC may be attenuated.

Blood profiles of ICC are presented in Table 3. The values of erythrocytes, hemoglobin, hematocrits, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width-coefficient variation (RDW-CV), mean platelet volume (MPV), platelet distribution width (PDW), leukocytes, heterophils, lymphocytes and thrombocytes were not affected ($p>0.05$) by dietary supplementation of *S. platensis* or combination of *S. platensis* and *S. cerevisiae*. In contrast to our findings, Opoola et al. (2019) showed that treatment with *S. platensis* (6, 12 or 18 g/kg feed) increased PCV, hemoglobin, and leukocytes in broiler chickens. Sugiharto et al. (2018), on the other hand, found that supplementing modern broiler chickens with *S. platensis* (1% of feed; throughout life) reduced the levels of hemoglobin, erythrocytes, hematocrits, leukocytes, and lymphocytes. In respect to *S. cerevisiae*, Lawrence-Azua et al. (2018) found that dietary *S. cerevisiae* supplementation raised PCV, leukocytes, heterophils, monocytes, and eosinophils, but decreased MCHC in broiler chickens. Differences in chicken breeds and strains, feed compositions, additive doses, and other experimental circumstances appeared to explain the above discrepancies.

There was a tendency ($p=0.07$) that coliform bacteria counts were lower in the ileum of SP than that in CONT chicks (Table 4). Likewise, the *Enterobacteriaceae* population tended ($p=0.07$) to decrease in the caeca of SPSC than that in other groups. The treatments did not have any impact on the pH values of intestinal segments of the ICC. Reduced coliform levels in the ileum of *S. platensis*-treated ICCs appeared to be linked to the microalgae's antibacterial activities (Abdel-Moneim et al. 2021). Like *S. platensis*, *S. cerevisiae* has also been shown to have antibacterial action (Fakruddin et al. 2017). As a result, the decreased *Enterobacteriaceae* counts in the caeca of chicks fed a mixture of *S. platensis* and *S. cerevisiae* may be explained.

Table 2. Performances of the ICC

Items	CONT	SP	SPSC	SE	p value
Week 4					
Final BW, g	268	283	286	3.43	0.06
Body weight gain, g	231	246	249	3.43	0.06
Feed intake, g	336	327	364	3.89	0.07
FCR	1.46^{a}	1.33^{b}	1.47^{a}	0.02	0.01
Week 9					
Final BW, g	817^{b}	872^{a}	843^{ab}	8.19	0.01
Body weight gain, g	780^{b}	835^{a}	806^{ab}	8.19	0.01
Feed intake, g	2231	2262	2236	15.6	0.71
FCR	2.87	2.71	2.78	0.04	0.29

Note: ^{a,b} Means marked by different superscript letters within the same row differ significantly ($p<0.05$); BW: body weight, FCR: feed conversion ratio, CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means

Table 3. Blood profiles of the ICC

Items	CONT	SP	SPSC	SE	p value
Erythrocytes (10^{12}/L)	3.14	4.12	3.74	0.19	0.11
Hemoglobin (g/dL)	12.0	15.5	14.1	0.77	0.18
Hematocrits (%)	37.3	48.8	44.4	2.33	0.13
MCV (fl)	119	119	119	0.44	0.98
MCH (pg)	37.9	37.5	37.5	0.35	0.90
MCHC (g/dL)	32.0	31.7	31.7	0.25	0.86
RDW-SD (10^{-15} L)	49.9	50.8	51.4	0.55	0.52
RDW-CV (%)	11.1	11.3	11.4	0.11	0.47
MPV (10^{-15} L)	8.93	9.03	8.70	0.11	0.49
PDW (%)	7.38	8.56	8.98	0.49	0.39
Leukocytes (10^9/L)	160	203	182	10.5	0.26
Heterophils (10^9/L)	14.4	17.0	17.4	1.42	0.66
Lymphocytes (10^9/L)	145	186	164	9.67	0.24
Thrombocytes (10^9/L)	12.9	14.3	16.7	0.81	0.16

Note: MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW-SD: red blood cell distribution width-standard deviation, RDW-CV: red blood cell distribution width-coefficient variation, MPV: mean platelet volume, PDW: platelet distribution width, CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means

Table 4. pH values and bacterial populations of intestine of the ICC

Items	CONT	SP	SPSC	SE	p value
pH values					
Duodenum	6.87	6.87	7.11	0.09	0.46
Jejunum	6.20	6.28	6.32	0.09	0.88
Ileum	5.98	6.47	6.59	0.15	0.24
Caecum	6.66	7.22	7.28	0.15	0.18
Bacterial counts in ileum (log cfu/g)					
Coliform	7.95	7.09	7.45	0.16	0.07
LNE	8.22	7.58	7.59	0.21	0.38
Enterobacteriaceae	8.45	7.58	7.88	0.21	0.24
LAB	11.2	11.0	11.2	0.07	0.42
LAB/coliform ratio	1.41	1.55	1.52	0.28	0.12
Bacterial counts in caecum (log cfu/g)					
Coliform	8.86	8.96	8.39	0.14	0.20
LNE	8.24	8.86	7.98	0.24	0.32
Enterobacteriaceae	8.87	9.45	8.64	0.15	0.07
LAB	11.5	11.7	11.7	0.06	0.27
LAB/coliform ratio	1.31	1.30	1.40	0.03	0.19

Note: CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means, LNE: lactose-negative Enterobacteriaceae, LAB: lactic acid bacteria, cfu: colony forming unit

The data on intestinal morphology of ICC are outlined in Table 5. It was apparent that dietary supplementation of *S. platensis* or a combination of *S. platensis* and *S. cerevisiae* had no substantial effect on the villi height, crypt depth and villi height to crypt depth ratio across the small intestinal segments of ICC. In contrast to our findings, dietary supplementation of *S. platensis* at 1, 1.5, or 2% of feeds increased the villi height, crypt depth, and villi height to crypt depth ratio of the duodenum, jejunum, and ileum of broiler chickens (Ansari et al. 2018). In laying hens, Mardanpour et al. (2021) found no significant effect of *S. platensis* on villi height, villi height to crypt depth ratio, and villi surface. The authors also stated that the inconsistent results of *S. platensis* treatment could be explained by the diverse types of chickens used for study, doses, and the nature of *S. platensis*. In the case of *S. cerevisiae*, the treatment with such live yeast yielded divergent results. *Saccharomyces cerevisiae* enhanced villus height in the jejunum and ileum in the study of He et al. (2021), but Julendra et al. (2021) reported no effect of dietary *S. cerevisiae* supplementation on the villus height of broiler jejunum.

It was shown in this present study that internal organ relative weight (Table 6) and carcass traits (Table 7) of ICC were not affected ($p>0.05$) by the dietary treatments.

Table 5. Intestinal morphology of the ICC

Items	CONT	SP	SPSC	SE	p value
Duodenum					
Villi height (µm)	1064	937.4	1015	40.9	0.47
Crypt depth (µm)	217	209	212	10.5	0.95
Villi height/crypt depth	5.15	4.59	5.17	0.34	0.76
Jejunum					
Villi height (µm)	978	987	878	50.8	0.65
Crypt depth (µm)	209	215	204	11.3	0.94
Villi height/crypt depth	4.82	4.77	4.28	0.25	0.64
Ileum					
Villi height (µm)	548	511	491	24.8	0.66
Crypt depth (µm)	108	127	148	10.1	0.29
Villi height/crypt depth	5.55	4.17	3.68	0.38	0.12

Note: CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means

Table 6. Internal organs relative weight of the ICC

Items (% live BW)	CONT	SP	SPSC	SE	p value
Heart	0.43	0.44	0.48	0.02	0.49
Liver	2.13	2.11	2.41	0.07	0.13
Proventriculus	0.58	0.54	0.51	0.03	0.55
Gizzard	3.53	3.16	3.47	0.10	0.30
Pancreas	0.24	0.22	0.30	0.02	0.38
Duodenum	0.80	0.72	0.78	0.05	0.82
Jejunum	1.45	1.40	1.45	0.11	0.98
Ileum	0.89	0.82	0.93	0.06	0.79
Caeca	0.75	0.63	0.66	0.03	0.30
Abdominal fat	0.32	0.49	0.38	0.09	0.77
Spleen	0.23	0.22	0.20	0.02	0.78
Thymus	0.48	0.49	0.45	0.04	0.87
Bursa of fabricius	0.14	0.17	0.14	0.02	0.83

Note: BW: body weight, CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means, BW: body weight

Table 7. Carcass traits of the ICC

Items	CONT	SP	SPSC	SE	p-value
Eviscerated carcass (% live BW)	56.1	56.4	57.1	0.68	0.86
	% eviscerated carcass				
Breast	25.4	24.9	24.3	0.47	0.62
Wings	16.3	15.7	15.6	0.23	0.41
Thigh	16.3	17.1	17.8	0.32	0.18
Drumstick	17.4	17.6	18.2	0.29	0.60
Back	24.5	24.7	24.2	0.23	0.75

Note: CONT: chicks provided with basal diet, SP: diet supplemented with 0.3% *Spirulina platensis*, SPSC: diet supplemented with 0.3% *Spirulina platensis* and 0.2% *Saccharomyces cerevisiae*, SE: standard error of the means, BW: body weight

Consistent with our findings, Sugiharto et al. (2018) found no effect of *S. platensis* on relative organ weight (excluding caeca) and carcass proportions of broiler chickens. Similarly, feeding *S. cerevisiae* had no effect on the relative weight of internal organs (excluding proventriculus) and the eviscerated carcass of broiler chickens (Sugiharto et al. 2019). Unlike the previous investigations, Kaoud (2012) found that feeding *S. platensis* and MOS derived from *S. cerevisiae* had a significant influence on absolute organ weights in broiler chicks. He discovered that feeding *S. platensis* to broilers lowered the absolute weight of the gizzard and liver, while feeding MOS reduced the absolute weight of the proventriculus and gizzard. The organ relative weights in our study were, however, not comparable to those published by Kaoud (2012) as he measured absolute organ weight. Indeed, assessing absolute organ weight may be biased by the fact that larger birds may have higher absolute organ weight. In contrast to Sugiharto et al. (2018) and our current finding, Kaoud (2012) documented that feeding *S. platensis* (but not MOS) increased the carcass percentage of broiler chickens. In such case, the high content of protein and growth-promoting properties of *S. platensis* (Islam et al. 2021; Moustafa et al. 2021) appeared to contribute to growth promotion or muscle protein accretion, resulting in an increase in the carcass proportion of chickens. In our study, the higher final body weight of SP-treated chicks was not accompanied by a higher carcass percentage of the chickens. In general, the disparity results about the carcass percentage have been attributed to variances in chicken types (modern broiler *versus* native or crossbred chickens), age, sex, physical activity and environmental condition of chickens (Li et al. 2017; Cygan-Szczegielniak and Bogucka 2021).

It can be concluded from the present study that dietary supplementation of *S. plantesis* improved growth performance and bacterial population of the ICC. Combining of *S. platensis* and *S. cerevisiae* did not exert synergistic effect on growth and health of ICC.

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