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RESEARCH ARTICLE

# Effects of *Saccharomyces cerevisiae* and *Spirulina platensis* on Growth Performances and Biochemical Parameters in Rabbits [1]

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## Summary

A feeding trial was conducted to evaluate the effect of live yeast culture *Saccharomyces cerevisiae* (SC) and *Spirulina platensis* (SP) on the growth performance and serum biochemical parameters in rabbits. Forty, male New Zealand white rabbits, aged 5-6 weeks, were studied in 4 groups. The groups; I. Control (basal diet), II. SC (added 3 g/kg diet), III. SP (added 5% of the diet), IV. SC and SP (added 3 g/kg SC and 5% SP of the diet), respectively. The experiment lasted for 90 days. Blood samples were obtained by ear venipuncture on the 90$^{th}$ day. Also final body weight, total weight gain, total feed intake and feed conversion ratio were evaluated at the each month of the 90$^{th}$ day trial. There were no significant differences occurred in growth performances and biochemical parameters, but serum globulin value decreased and albumin globulin ratio increased in SP and SC+SP groups ($P<0.05$). More studies would be necessary to elucidate the effects of supplementing spirulina on growth and determine the optimum dietary concentration in animals.

***Keywords:*** *Rabbits, Saccharomyces cerevisiae, Spirulina platensis, Growth performance, Serum biochemical parameters*

# Tavşanlarda *Saccharomyces cerevisiae* ve *Spirulina platensis*'in Büyüme Performansı ve Serum Biyokimyasal Parametreleri Üzerine Etkileri

## Özet

Bu araştırma canlı maya kültürü *Saccharomyces cerevisiae* (SC) ve *Spirulina platensis* (SP)'in tavşanlarda büyüme performansı ve serum biyokimyasal parametreleri üzerine etkilerini değerlendirmek için yapıldı. 40 tane, 5-6 haftalık beyaz Yeni Zelanda tavşanı 4 gruba ayrıldı. Gruplar sırasıyla; I. Kontrol (bazal diyet), II. SC (3 g/kg yeme ilave edildi), III. SP (%5 oranında yeme ilave edildi), IV. SC ve SP (SC 3 g/kg ve SP %5 oranında yeme ilave edildi) olarak belirlendi. Çalışma 90 gün sürdü. 90. gün sonunda kulak venasından kan örnekleri toplandı. Ayrıca, doksan günlük çalışmada her ay, son canlı ağırlık ile toplam canlı ağırlık artışı ve toplam yem tüketimi ile yemden yaralanma değerlendirildi. Büyüme parametreleri ve biyokimyasal parametrelerde önemli farklılık bulunamadı fakat SP ve SC+SP gruplarında serum globulin değeri azaldı ve albumin-globulin oranı ise arttı ($P<0.05$). Spirulina ilavesinin büyüme performansı üzerine olan etkilerini aydınlatmak ve hayvanlarda en uygun diyet konsantrasyonunu belirlemek için daha fazla çalışmaya gereksinim vardır.

***Anahtar sözcükler:*** *Tavşan, Saccharomyces cerevisiae, Spirulina platensis, büyüme performansı, Serum biyokimyasal parametreleri*

## INTRODUCTION

Single cell proteins like *Saccharomyces cerevisiae* (SC) and *Spirulina platensis* (SP) are considered as alternative protein sources in animal diets. These natural additives have been observed in studies of fish [1], cattle [2], pigs [3], chickens [4-6] and rabbits [7-9].

The yeast *S. cerevisiae* is a well-known probiotic having positive effects in the treatment and prevention of diseases [10]. The positive effect of probiotic can originate from either their direct nutritional effect or their health promoting effects such as acting as a bioregulator of the intestinal microflora and reinforcing the host's natural defenses [11]. *S. cerevisiae* contains biologically valuable proteins, vitamin B-complex, important trace minerals and several unique "plus" factors. First, Eckles and Williams [12] reported the use of *S. cerevisiae* as a growth promoter for

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ruminants. The inclusion of live yeast into animal feed has been shown to improve the digestibility, efficiency of feed utilization, and performance of animals [13,14]. The addition of Saccharomyces cerevisiae had a growth stimulating effect and generally, the responses were linearly related to the concentration of yeast [7]. Onifade et al.[7] studied with SC level at 1.5 g/kg and 3.0 g/kg, and they observed the positive effect on weight gain, feed intake and feed conversation ratio in rabbits. Eze and Ezema [15] suggested that SC in level of 0.12 g/kg of diet had a beneficial effect on growth and health status of rabbit. *S. cerevisiae*. is also called as single cell protein [11,16], has been shown to survive in the gastrointestinal tract while eliminating the potential pathogenic bacteria residing in channel [17]. Also, Kimsé et al.[18] was found that the survival rate of yeast in digestive tract was higher in rabbits. However, this addition did not affect the feed intake, feed efficiency and final body weight of rabbits.

It has been suggested that probiotics binds to bile acids which results in a reduced serum cholesterol value [19]. There are many conflicting studies about the effect of *S. cerevisiae* on serum lipid profile in animals. Although some of the studies showed reduction in cholesterol [5,16,20], the others demonstrated no benefits [11,21].

*S. platensis* is a microscopic filamentous alga, which contains several vitamins, especially vitamin $B_{12}$ and pro-vitamin A (beta-carotene). Also, it is rich in polyunsaturated fatty acids, phycocyanin [22,23] and phenolic compounds [24]. Over the years, many dietary supplements of *S. platensis* have a widespread use. *S. platensis* has been approved as a health food by the World Health Organization (WHO) and it will become one of the most alternative treatments in the 21st century [25,26]. Researchers have reported the effects of *S. platensis* on blood protein and lipid content [23,27,28], and their antioxidant, antiviral and immunmodulator activities [24,29,30] in animals. According to some researchers, *S. platensis* and its extract may decrease the blood lipid values. Especially, phycocyanin and polyunsaturated fatty acids in *S. platensis* may play an important role in its hypocholesterolemic effect [28,29,31]. Nagaoka et al.[23] reported that cholesterol is lowered by inhibition of the cholesterol absorption from jejunum and bile acid resorption from ileum with phycocyanin in *S. platensis*. In addition, other researchers proposed that *Spirulina platensis* may have an effect on the increments in plasma total protein, albumin and globulin values [6,32] in animals. These researchers stated that the increased concentrations of plasma total protein, albumin and globulin may be related to the high protein contents in *S. platensis* (with values ranging from 55-65% and includes all of the essential amino acids).

Contradictory results are available in the literatures of dietary Spirulina effects on the growth performances in rabbits [33-35]. Dalle Zotte et al.[33] studied with female rabbits which reached a higher body weight due to high feed intake with *S. platensis* addition. The efficiency of *S. platensis* for rabbit growing has been tested by Peiretti and Meineri [34]. They showed that the final weight. weight gain and feed efficiency did not differ significantly among the dietary treatments, but *S. platensis* inclusion at a level of 10% gave the highest feed intake. Similary, Gerencsér et al.[35] had found no statistical differences for final weight and weight gain.

Although some studies have been performed in the performance of *S. cerevisiae* and *S. platensis* in animals [5,16,23], the combined effect of *S. cerevisiae* and *S. platensis* have not been addressed yet. This research was aimed to evaluate the combined effect of *S. cerevisiae* and *S. platensis* on growth performance and biochemical parameters. Also, there is not enough data about the effects of *S. platensis* in animals. So, this study contributes to an understanding of the literature about *S. platensis*.

## MATERIAL and METHODS

### *Animals, Groups and Feeding*

Forty male New Zealand white rabbits aged 5-6 weeks with 1000.9 g mean body weight were randomly allocated on a weight basis to four groups: I. Control, II. SC (added 3 g/kg diet), III. SP (added 5% of the diet), IV. Combination of SC and SP (added 3 g/kg SC and 5% SP of the diet), respectively. The rabbits were housed individually in metal cages and provided with separate facilities for feeding and watering. Feed and water were offered *ad libitum* to the rabbits throughout the 90 day trial. Basal diet (pelleted) was formulated to contain 2.500 kcal ME/kg metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the NRC [36]. Chemical composition and ingredients of the diet are provided in *Table 1* and *Table 2*. Chemical analyses of diets were carried out according to AOAC [37]. Basal diet was supplemented with *S. cerevisiae* live yeast culture (Yea Sacc$^{1026}$ Altech. Nicholasville: 1x10$^{9}$ CFU g$^{-1}$) and/or *Spirulina platensis*.

The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The

***Table 1.*** *Chemical composition of basal diet (%DM)*
***Tablo 1.*** *Bazal diyetin kimyasal içeriği (%Kuru Madde)*

| Chemical Analysis | Diet |
|---|---|
| Dry matter % | 88.89 |
| Crude protein %* | 16.00 |
| Ether extracts %* | 3.52 |
| Crude fiber %* | 10.95 |
| Ash | 7.68 |

** Based on % dry matter*

**Table 2.** *Ratio of feed ingredients (%)*
**Tablo 2.** *Yem içeriği oranları (%)*

| Ingredients | Usage Rate % |
|---|---|
| Barley | 30.00 |
| Corn14 | 17.61 |
| Rice bran | 10.00 |
| Corn bran | 3.60 |
| Alfalfa meal | 25.00 |
| Soybean meal 46 | 10.83 |
| Limestone | 1.40 |
| Dicalcium phosphate 18 | 0.28 |
| Salt | 0.80 |
| Methionine | 0.09 |
| Anticoccidial | 0.03 |
| Vitamin premix* | 0.25 |
| Antioccidial | 0.03 |
| Total | 100.00 |

** **Premix:** Vit A 4.800.000 IU, Vit D 800.000 IU, Vit E 14.000 mg, Biotin 18 mg, CH-CL 50.000 mg, Folic acid 400 mg, Niacin 8.000 mg, Pant. acid 4.000 mg, Riboflavin 2.800 mg, Thiamin 1.200 mg, Pyridoxine 2.000 mg, Vit K 1.600 mg, Zinc 24.000 mg, Iron 2.000 mg, Iodine 400 mg, Manganese 32.000 mg, Selenium 60 mg, Copper 24.000 mg*

study was carried out with the permission of Uludag University Animal Experimentation Local Ethics Committee (Approval No: 2010-09/01).

### *Measurements*

Initial body weight, final body weight, total body weight gain, total feed intake and feed conversion ratio of each rabbit were determined for growth performance in the each month of the 90$^{th}$ day trial. Blood samples were collected for non-anticoagulant tubes by ear venipuncture on the 90$^{th}$ day from overnight-fasted rabbits. Serum concentrations of total protein, albumin, globulin, total lipid, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose, creatinine, creatine kinase, amylase, lipase, urea, sodium, potassium, phosphorus, calcium, ferrous and activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined by "Clima MC15" auto analyzer (RAL, Barcelona, Spain).

### *Statistical Analysis*

Statistical analyses were performed with SPSS [38] (Version 17.0; Chicago.IL). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at $P<0.05$. If the difference between groups was provided to be significant ($P<0.05$), differences evaluated by Tukey's test [39]. On the other hand, in non-homogenous groups, differences between means were analyzed by Kruskal Wallis and following Mann Whitney U test between groups one by one [40].

## RESULTS

Performance characteristics of the control and experimental groups (SC, SP and SC+SP) are presented in *Table 3*. There were no significant changes in the growth performances of the groups, monthly or at the end of the experiment. Nevertheless there were no significant changes in the serum biochemical indices, as shown in *Table 4*; only the serum globulin value was significantly ($P<0.05$) lower and albumin-globulin ratio was significantly higher in the SP and SC+SP groups compared to the Control and SC groups. Also, data on serum cholesterols, enzymes and minerals are summarized in *Table 4*. There were no changes in these parameters ($P>0.05$).

## DISCUSSIONS

*S. cerevisiae* and *S. platensis* have received attention as a good probiotic and prebiotic organism that can maintain growth performance characteristics, and also associated with health promoting effects. The present study is an attempt to identify natural effects of SP, SC and SP+SC combinations for rabbits. According to the results of this study, although not statistically significant, supplementing rabbit with a combination of *S. cerevisiae* and *S. platensis* had increased on mean body weight and body weight gain. The monthly body weight gain, feed intake and feed conversation ratio of rabbits were not significantly affected by SC, SP and combination of SC and SP. In addition to, the feed conversation ratio was affected positively in the group of feeding *S. platensis*.

The effect of *S. platensis* on growth performance values in animals was determined by some researchers [2,41,42]. Heidarpour et al.[2] studied with 3 levels of *S. platensis* (2, 6 and 25 g) in Holstein calves and they reported that there were no significant differences in weight gain, daily feed intake and feed conversion ratio. Also, Dernekbasi et al.[42] added 10%, 20%, 30% and 40% *S. platensis* into diet of fish and determined no effect on the growth parameters among groups. However, there are some reports indicating a significant increase in the body weight gain by dietary *S. platensis* supplementation [1,41,44]. In the present study, there were no significant differences in growth performances among the groups, but mean feed conversion ratio was slightly lower in the *S. platensis* group than the other groups. Similar to our results, Peiretti and Meineri [34] observed no significant differences in growth performances in rabbits fed with *S. platensis* supplementation. The mechanisms of growth promotion of yeast culture in rabbits, turkey poults and broiler chickens and the positive relationship between *S. cerevisiae* and animal performance characteristics have

***Table 3.*** *Growth performances characteristics of rabbits in control and experimental groups (mean±standard error, n=40)*
***Tablo 3.*** *Kontrol ve deney gruplarının büyüme performansı değerleri (ortalama±standart hata, n=40)*

| Traits | Control n=10 | SC n=10 | SP n=10 | SC+SP n=10 |
|---|---|---|---|---|
| Initial body weight (g) | 1009.20±73.94 | 1009.56±76.15 | 1009.20±75.45 | 1009.20±78.57 |
| Final body weight (g) | 2662.40±48.37 | 2717.78±83.42 | 2677.00±80.01 | 2718.80±100.50 |
| Total weight gain (g) | 1653.20±83.78 | 1680.22±69.89 | 1695.20±82.56 | 1709.60±77.93 |
| Total feed intake (g) | 7785.40±236.97 | 8431.33±143.90 | 7443.40±292.25 | 7815.80±317.52 |
| Total feed conversion ratio | 4.79±0.23 | 5.08±0.22 | 4.48±0.25 | 4.60±0.16 |
| **First Month (0-30 days)** | | | | |
| Live weight (g) | 1771.60±44.61 | 1859.56±63.03 | 1847.60±63.52 | 1824.20±82.00 |
| Body weight gain (g) | 762.40±47.98 | 822.00±48.31 | 838.40±44.01 | 815.00±32.55 |
| Feed intake (g) | 2181.60±99.89 | 2400.00±56.95 | 2119.00±71.63 | 2179.80±137.88 |
| Feed conversion ratio | 2.99±0.27 | 2.99±0.18 | 2.60±0.18 | 2.67±0.10 |
| **Second Month (30-60 days)** | | | | |
| Live weight (g) | 2353.60±38.17 | 2398.44±83.07 | 2386.60±70.12 | 2398.80±95.18 |
| Body weight gain (g) | 582.00±23.81 | 538.89±37.21 | 539.00±51.05 | 574.60±46.54 |
| Feed intake (g) | 2990.00±81.09 | 3377.78±116.04 | 2862.00±151.21 | 2954.20±173.37 |
| Feed conversion ratio | 5.19±0.20 | 6.66±0.77 | 5.63±0.45 | 5.40±0.43 |
| **Third Month (60-90 days)** | | | | |
| Live weight (g) | 2662.40±48.37 | 2717.78±83.42 | 2677.00±80.01 | 2718.80±100.50 |
| Body weight gain (g) | 308.80±26.50 | 319.33±30.13 | 317.80±30.46 | 320.00±23.70 |
| Feed intake (g) | 2853.80±152.88 | 2986.89±142.84 | 2552.40±142.01 | 2861.80±99.18 |
| Feed conversion ratio | 9.63±0.66 | 10.21±1.23 | 8.68±0.91 | 9.30±0.62 |

***Table 4.*** *Serum biochemical indices of rabbits in control and experimental groups. (mean±standard error, n=40)*
***Tablo 4.*** *Kontrol ve deney gruplarının serum biyokimyasal değerleri (ortalama±standart hata, n=40)*

| Biochemical Parameters | Control | SC | SP | SC+SP |
|---|---|---|---|---|
| Total protein (g/dl) | 6.78±0.27 | 6.60±0.48 | 6.13±0.16 | 6.30±0.22 |
| Albumin (g/dl) | 3.56±0.20 | 3.90±0.18 | 3.91±0.14 | 4.13±0.92 |
| Globulin (g/dl) | 3.16±0.22$^{a}$ | 3.27±0.25$^{a}$ | 2.31±0.13$^{b}$ | 2.29±0.21$^{b}$ |
| Albumin/Globulin | 1.19±0.12$^{a}$ | 1.27±0.12$^{a}$ | 1.75±0.13$^{b}$ | 1.95±0.18$^{b}$ |
| Total lipid (mg/dl) | 378.50±7.90 | 385.78±3.55 | 364.70±7.31 | 375.70±7.11 |
| Triglyceride (mg/dl) | 119.40±8.24 | 114.89±10.71 | 113.40±10.71 | 107.10±7.97 |
| Cholesterol (mg/dl) | 53.60±7.87 | 62.67±8.12 | 63.00±10.86 | 77.00±8.97 |
| HDL cholesterol (mg/dl) | 31.60±4.40 | 30.11±3.18 | 38.40±5.65 | 37.90±5.78 |
| LDL cholesterol (mg/dl) | 15.25±1.43 | 17.37±328 | 16.92±2.34 | 21.02±5.36 |
| LDL/HDL ratio | 0.59±0.10 | 0.64±0.15 | 0.50±0.09 | 0.58±0.15 |
| Urea (mg/dl) | 40.90±2.77 | 37.89±4.30 | 38.70±2.19 | 34.90±1.72 |
| Glucose (mg/dl) | 138.80±6.23 | 167.67±15.69 | 133.9±8.23 | 130.70±6.94 |
| Creatinine (mg/dl) | 0.84±0.08 | 0.80±0.09 | 0.81±0.06 | 0.92±0.05 |
| Creatinine kinase (U/L) | 602.80±196.96 | 556.56±125.89 | 473.70±115.80 | 310.40±51.70 |
| Amylase (U/L) | 509.40±42.68 | 425.56±35.01 | 518.10±40.50 | 454.10±29.02 |
| Lipase (U/L) | 153.60±6.44 | 163.55±11.95 | 147.50±9.32 | 156.00±4.49 |
| Phosporus (mg/dl) | 6.23±0.24 | 5.69±0.41 | 6.34±0.37 | 5.85±0.32 |
| Calcium (mg/dl) | 12.52±0.61 | 10.73±0.58 | 10.47±0.74 | 10.38±0.62 |
| Sodium (mmol/l) | 159.30±4.14 | 167.44±9.18 | 151.80±8.88 | 171.50±3.72 |
| Ferrous (mmol/l) | 56.19±2.65 | 51.92±4.45 | 43.55±5.92 | 55.84±4.32 |
| Potassium (mg/dl) | 4.70±0.21 | 4.56±0.24 | 4.61±0.15 | 4.55±0.20 |
| GOT (U/l) | 33.30±6.45 | 40.67±5.62 | 36.10±7.03 | 45.90±8.21 |
| GPT (ALT) (U/l) | 56.80±11.20 | 61.56±6.29 | 52.00±8.70 | 66.70±10.82 |
| ALP (U/l) | 427.00±29.92 | 369.67±28.84 | 402.50±23.87 | 398.60±31.16 |

*Different superscripts a, b, show differences (P<0.05) between groups*

been reported [4,5,43,45]. Onifade and Babuntae [4] studied in broiler chicks with a diet containing 6.0 g/kg *S.cerevisiae* yeast. They interpreted that yeast may effect on improving feed quality. On the contrary, Lambertini et al.[45] and Chaudhary et al.[43] reported that yeast did not affect live weight, daily weight gain and feed intake in New Zealand rabbits. Also, in our study, growth parameters were not affected by the addition of the SC *(Table 3)*.

*S. platensis* and *S. cerevisiae* are used as a supplement because of their protein, vitamin and mineral content. In the present study, serum globulin value was significantly lower, and therefore albumin-globulin ratio was significantly higher in groups fed SP and SC+SP compared to the control and SC groups (*Table 4*, P<0.05). Globulin fractions were not determined in this study, so we do not know the source of the decline in the value of globulin. However,

addition of SP may inhibit the growth of harmful bacteria in intestine [46] and the lower value of serum globulin of rabbits fed at 5% *S. platensis* may be attributed to the inhibitory effect of *S. platensis* against harmful intestinal microflora. Because harmful enteric bacteria secretes inflammatory agents and lead to increase in globulin synthesis of liver or of other tissues such as lymphatic tissue or plasma cells. *S. cerevisiae* has also an inhibitory effect against harmful intestinal microflora [11], but the effects of *S. cerevisiae* and *S. platensis* on immune response may be different from each other. On the other hand, Heidarpour et al.[2] studied on albumin, globulin and their assigned ratio in calves feeding *S. platensis* in levels of 0, 2, 6 and 25 g/day, and found no significant effect on serum albumin and globulin levels among treatment groups. Also, Moreira et al.[41] found no significant effect of *S. platensis* on serum albumin and protein levels. However, Mariey et al.[6] stated that SP level at 0.2% had a significant increase in plasma total protein, albumin and globulin in laying hens. Bezeria et al.[32] determined the high serum protein value in lambs fed 0, 5 and 10 g SP. These researchers suggested that the high value of serum protein, globulin and albumin may be due to protein quality and quantity of *S. platensis*.

*S. cerevisiae* cell wall component, beta glucan, had a cholesterol lowering effect was documented by some researchers [16,19,20]. Paryad and Mahmoudi [16] reported reduction in plasma cholesterol and triglyceride value of chicks which was fed with yeast supplement. These researchers suggested that yeast may regulate the serum cholesterol concentrations by deconjunction of bile acids. However, Payandeh [47], Ozsoy et al.[48], and Yildiz et al.[49] observed that SC did not affect the serum lipid, triglyceride and cholesterol. Also in the present study, there were no changes in serum lipid, triglyceride and cholesterol values of rabbits fed with the *S. cerevisiae* supplement ($P>0.05$, *Table 4*).

*S. platensis* is rich in polyunsaturated fatty acids and phycocyanin [22-24]. Some researchers [31,50,51] reported that the polyunsaturated fatty acids in *S. platensis* help to reduce serum lipid profiles. The addition of 16% of *S. platensis* into the rat diet caused to significant inhibition of serum total cholesterol in the study accomplished by Kato et al.[50]. Also, Nagaoka et al.[23] reported that phycocyanin plays a crucial role in the hypocholesterolemic action of *S. platensis* concentrate in rats. These researchers suggested that phycocyanin may inhibit both jejunal cholesterol absorption and ileal bile acid reabsorption by binding bile acid. However, in the present study there were no changes in these parameters of rabbits fed with *S. platensis* supplementation.

Average values of aspartate aminotransferase (AST), alanin aminophosphatase (ALP) and alanine amino-transaminase (ALT) in serum of rabbits were revealed no statistically significant differences between control. SC, SP and SC+SP groups *(Table 4)*. These enzymes are located intracellularly in the body including liver, heart and kidney etc. Their level in the blood is increased when there is membrane damage in these cells. Hence, normal level of these enzymes in blood of rabbits fed diet containing 3 g/kg SC and 5% SP suggests that it has no adverse effect on the cells of vital organs. These results agree with results of Shrivastava and Jha [20], Saied et al.[52], Ibrahim et al.[53], Moreira et al.[41] and Sixabella et al.[54].

Administration of *S.cerevisiae* and *S. platensis* singly or in combination had no significant effect on serum biochemical parameters, except for serum globulin and albumin-globulin ratio. These results don't indicate that these supplements have any positive effects. However, there was a slightly positive effect of *S. platensis* on growth parameters, especially on feed conversion ratio. On the other hand, although not statistically significant, *S. platensis* and *S. cerevisiae* combination improved the body weight and body weight gain compared to control. So, *S. platensis* may be used as a growth promoter in animals. However, more studies would be necessary to elucidate the effects of supplementing spirulina on growth and to determine the optimum dietary concentration in animals.

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