



Autolyzed *Saccharomyces cerevisiae* as a single cell protein for broiler diet

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Abstract

Microbial protein often called as single cell proteins (SCP) are becoming a potential alternative to conventional protein rich ingredients in poultry diet. An experiment was conducted to know the possibility of using *Saccharomyces cerevisiae* derived SCP in broiler diet. A total of 96 male Ross-308 day old chicks were assigned to four diet comprising control (0% replacement), 25% replacement, 50% replacement and 100% replacement of protein concentrate with autolyzed *S. cerevisiae*. Each group had 6 replicates of 4 chicks in each. Body weight, body weight gain, daily weight gain, feed intake and feed conversion ratio were not affected ($P>0.05$) in case of 25% and 50% replacements. However, 100% replacement had resulted numerically negative effect as compared to control group. Dressing percentage and other carcass characteristics were not affected ($P>0.05$) in the replacement groups. The results of this study indicated that autolyzed *S. cerevisiae* might be used to replace protein concentrate at 25% to 50% level in broiler diet without affecting growth and carcass quality.

Key words: *Saccharomyces cerevisiae*, protein concentrate, broiler, single cell protein

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Introduction

Commercial poultry farming is playing a vital role in providing cheap animal protein to human diet in Bangladesh. However, most of the inputs for commercial poultry farming are of imported origin (Chowdhury, 2013). Thus, Bangladesh is spending substantial amount of foreign currency every year to import feed ingredients like protein concentrates and soyabean meal. This situation is creating a demand for the formulation of innovative and alternative proteinaceous feed sources for livestock and poultry. Single cell protein (SCP) production might be a major step in this direction. SCP is the protein extracted from cultivated microbial biomass (Nasseri *et al.*, 2011). It can be used for protein supplementation of a staple diet by replacing costly conventional sources like soymeal, protein concentrate and fishmeal. In addition, bio-conversion of agro industrial wastes to protein rich feed ingredients has an additional benefit of making the final product cheaper. This would also offset the negative cost value of wastes used as substrate to yield SCP. Further, it would make feed production less dependent upon land and relieve the pressure on agriculture. SCP are being produced from various microorganisms such as algae, bacteria, fungi and yeasts for both human and livestock.

Due to ease of propagation using cheap agro industrial byproducts and isolation; yeasts, specially *Saccharomyces cerevisiae* (*S. cerevisiae*) has become more convenient source of SCP (Ravindra, 2000; Bekatorou *et al.*, 2006; Nasseri *et al.*, 2011). To date, numerous potential strains of microbes and substrates that could be utilized for SCP production are proposed. However, nutritive value and removal of nucleic acids and toxins from SCP as a protein source is crucial before being considered a particular SCP for poultry diet.

S. cerevisiae is a good source of various nutrients like crude protein, amino acids, fatty acid, mineral elements, vitamin, etc., and could be a potential options for producing SCP (Yamada and Sgarbieri, 2005; Bacha *et al.*, 2011; Yassine *et al.*, 2013). SCP derived from *S. cerevisiae* contains 44.40% crude protein, 1% crude fat, 2.70% crude fiber, 0.12% Calcium and 1.40% Phosphorus and also 1,990 Kcal/Kg metabolizable energy (Shahryar *et al.*, 2012; Chand *et al.*, 2014). In addition, the cell wall fraction is rich in glycan and mannanoligosaccharides (MOS), which enhance growth of gut probiotic bacteria in poultry (Fernandez *et al.*, 2002). Use of *S. cerevisiae* as a protein source in poultry feed requires destruction of cell wall to release the cell contents. The destruction process can be

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accomplished by autolysis. The most common method of autolysis is heat treatment and changing the pH of the media. *S. cerevisiae*, either whole or autolyzed is being used as supplementation in broiler diets to improve different growth parameters (Gao *et al.*, 2008). However, the question of using autolysed *S. cerevisiae* as a replacement of commercial protein concentrate is still open. Thus, this study was conducted to replace commercial protein concentrate with SCP derived from autolyzed *S. cerevisiae* in broiler diet.

Materials and Methods

Production and autolysis of *S. cerevisiae* biomass

Molasses based media was used as the carbon source for mass production of *S. cerevisiae*. Sugarcane molasses was collected from the local market and 15% media was prepared with normal tap water and the Brix value was adjusted to 11°. The pH of the media was adjusted to 5.26. Commercial grade urea was added 6gL⁻¹ to increase nitrogen content of the media. 1.8L of previously prepared *S. cerevisiae* pure stock was inoculated to 350L growth medium following autoclave at 121°C and 15 PSI for 30 minutes and cooling. The fermentation process was

carried out within a customized bioreactor at 28-30°C for 72 hours. During the culturing process, 15 litre/minute purified air was supplied to the media to maintain aerobic condition. *S. cerevisiae* was harvested by gravitational method. Then the cells were washed with distilled water and autolyzed at 55°C for 36 hours. After autolysis, the autolyzed *S. cerevisiae* was kept at 80°C for 30 mins to stop the autolysis process as described by Tangüler and Erten (2009). Then the autolysate was dried at 60°C to remove moisture.

Feeding trial

Oven dried autolyzed *S. cerevisiae* was used to replace the protein concentrate at 25%, 50% and 100% in feed. Experimental diets were formulated to meet up the nutrients requirement of ROSS-308 using high quality commonly available feed ingredients. The diets were formulated for starter and grower phases (ingredients and nutritional composition are presented in **Table 1** and **Table 2**, respectively) and presented to the birds in mash form. The chicks were randomly assigned to different dietary groups in a completely randomized design (CRD). The duration of the experiment was 35 days.

Table 1. Ingredients and nutritional composition of starter diet (0-21 day)

Ingredients (%)	Level of replacement			
	0%	25%	50%	100%
Maize	51.0	49.30	48.0	45.80
Protein concentrate	5.0	3.75	2.50	0
Yeast autolysate	0	1.25	2.50	5.0
Soybean meal	37.60	38.80	39.70	41.60
Soybean oil	3.0	3.50	3.80	4.0
Di-Calcium Phosphate	2.50	2.55	2.70	2.80
L-Lysine	0.20	0.18	0.18	0.18
DL-Methionine	0.20	0.18	0.18	0.18
Vitamin premix*	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.20	0.20
Total	100	100	100	100
Diet compositions				
Crude protein (%)	22.58	22.57	22.49	22.45
Metabolizable energy (Kcal/Kg)	3010	3011	3001	2995
Calcium (%)	0.96	0.93	0.93	0.88
Available Phosphorus (%)	0.62	0.62	0.63	0.61
Lysine (%)	1.51	1.48	1.41	1.44
Methionine (%)	0.61	0.59	0.56	0.55

*Vitamin premix supplied per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 4,500 IU; vitamin E, 70 IU; vitamin K, 3 mg; vitamin B1, 3.2 mg; vitamin B2, 8.4 mg; vitamin B6, 4.1 mg; D-pantothenic acid, 20.0 mg; niacin, 65 mg; biotin, 0.25 mg; folic acid, 2 mg; vitamin B12, 0.02 mg.

Table 2. Ingredients and nutritional composition of grower diet (22-35 day)

Ingredients (%)	Level of replacement			
	0%	25%	50%	100%
Maize	55.7	54.6	52.9	50.1
Protein concentrate	5	3.75	2.5	0
Yeast autolysate	0	1.25	2.5	5
Soybean meal	32.6	33.35	34.45	36.15
Soybean oil	3.7	4.1	4.6	5.4
Di-Calcium Phosphate	2.1	2.2	2.3	2.6
L-Lysine	0.2	0.15	0.15	0.15
DL-Methionine	0.2	0.15	0.15	0.15
Vitamin premix*	0.25	0.25	0.25	0.25
Common salt	0.25	0.2	0.2	0.2
Total	100	100	100	100
Diet compositions				
Crude protein (%)	20.9	20.8	20.8	20.7
Metabolizable energy (Kcal/Kg)	3103	3104	3102	3098
Calcium (%)	0.86	0.84	0.83	0.83
Available Phosphorus (%)	0.5	0.5	0.5	0.6
Lysine (%)	1.39	1.32	1.26	1.28
Methionine (%)	0.59	0.53	0.51	0.50

*Vitamin premix supplied per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 4,500 IU; vitamin E, 70 IU; vitamin K, 3 mg; vitamin B1, 3.2 mg; vitamin B2, 8.4 mg; vitamin B6, 4.1 mg; D-pantothenic acid, 20.0 mg; niacin, 65 mg; biotin, 0.25 mg; folic acid, 2 mg; vitamin B12, 0.02 mg.

Management of experimental broilers

Day old chicks were collected from local commercial hatchery and individually identified by wing banding and randomly assigned into four dietary groups; control group (0%), three replacement groups (25%, 50% and 100%) with 24 chicks per group. The experiment was conducted at the open sided house of Bangladesh Agricultural University Poultry Farm. 5 cm deep rice husk was used as litter material. One chick feeder and drinker were provided in each pen. The feeders and drinkers were fixed in such a way that the broilers were able to eat and drink conveniently. Drinkers were washed and cleaned everyday while feeder were cleaned once in a week. Feed and fresh drinking water was supplied *ad-libitum* basis. Birds were vaccinated against Infectious Bronchitis (IB), New castle Disease (ND) and Infectious Bursal Diseases (IBD). Standard biosecurity measures were followed throughout the rearing period.

Data collection and record keeping

Weekly body weights of individual bird and weight of feed given and left over were measured and recorded. Live body weight (LBW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were calculated for each growth phases. At the end of the feeding trial, one broiler per replication having similar body weight was sacrificed and used for recording different carcass parameters. The birds were sacrificed and allowed to bleed completely and

scalded by immersing in hot water (51-55°C) for 2 min. The feathers were removed by hand pinning. Finally, records were kept on weight of dressed broilers, feather, blood, shank, liver, heart, gizzard, spleen, head, neck and intestine. The contents of gizzard were expelled to record its weight.

Statistical analysis

Collected data were analyzed using a linear model implemented in R (R Core Team, 2016). Effect of diets, individual and replication were treated as fixed effects. Diet effects on different body weight, growth and carcass parameters were compared with control diet by Dunnett's test.

Results

Body weight

Body weight (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae* is presented in **Table 3**. Insignificant difference ($P = 0.8580$) of initial body weight was found among the dietary groups. However, the effect of replacing protein concentrate with autolyzed *S. cerevisiae* was significant at 7, 14, 21 and 28 days of age. In contrary, the effect was insignificant ($P = 0.1150$) at 35 days of age. No significant differences in body weight was observed in 25% and 50% replacement group as compared to control group (0% replacement) at 7 days of age, whereas significantly ($P < 0.05$) lower body weight was

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observed in 100% replacement group as compared to control group. However, no significant difference in body weight was observed in 25% replacement group as compared to control group at 14 days; whereas significantly ($P<0.01$) lower body weights were observed in 50% and 100% replacement group. Similar trends of body weight were observed at 21 days of age in 25% replacement group as compared to control group, but significantly ($P<0.05$) lower body weights were observed in 50% and 100% replacement group.

At 28 days, 25% and 50% replacement group showed insignificant ($P>0.05$) difference in body weight as compared to control group while 100% replacement group showed significantly ($P<0.05$) lower body weight. At the age of 35 days, there was no significant difference in body weight among different dietary groups was found, but numerically lower value was observed in 100% replacement group which is statistically non-significant ($P = 0.1150$).

Table 3. Body weight (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae*

Age (days)	Level of replacement				P-value
	0%	25%	50%	100%	
0	48.44±2.10	48.89±2.04	49.10±1.97	48.94±1.39	0.8580
7	165.83±19.85	165.38±17.61 ^{NS}	149.17±17.37 ^{NS}	147.25±17.30*	1.18×10 ⁻²
14	381.38±58.16	371.92±50.72 ^{NS}	303.54±71.92**	302.38±44.00**	6.24×10 ⁻⁴
21	694.63±105.41	664.58±125.00 ^{NS}	583.58±134.58*	581.92±76.69*	0.0259
28	1022.33±146.06	1052.67±171.29 ^{NS}	948.71±248.78 ^{NS}	910.83±127.25*	0.0275
35	1654.09±166.65	1637.92±237.23	1510.58±325.04	1453.08±162.08	0.1150

Data are Mean ± SD. ^{NS}: non-significant, *: $P<0.05$, **: $P<0.01$ compared to control diet (0% replacement) as determined by Dunnett's test

Table 4. Body weight gain (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae*

Age (days)	Level of replacement				P-value
	0%	25%	50%	100%	
0-7	117.39±20.02	116.49±16.82 ^{NS}	100.07±17.31 ^{NS}	98.31±17.16*	0.0089
7-14	215.54±39.59	206.54±37.29 ^{NS}	154.38±59.06**	155.13±32.72**	6.93×10 ⁻⁵
14-21	313.25±60.61	292.67±79.62	280.04±74.15	279.54±50.61	0.5418
21-28	412.59±58.87	388.08±62.57	365.13±123.82	328.92±108.46	0.173
28-35	538.82±85.85	585.25±92.46	561.88±115.55	542.25±86.40	0.646
0-21	646.18±105.36	615.70±124.42 ^{NS}	534.49±134.54*	532.97±76.41*	0.0247
21-35	951.41±124.21	973.33±141.67	927.00±208.75	871.17±117.68	0.420
0-35	1605.92±166.34	1589.03±236.75	1461.49±324.99	1404.14±161.47	0.113

Data are Mean ± SD. ^{NS}: non-significant, *: $P<0.05$, **: $P<0.01$ compared to control diet (0% replacement) as determined by Dunnett's test.

Table 5. Feed intakes (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae*

Age (days)	Level of replacement				P-value
	0%	25%	50%	100%	
0-7	168.33±3.23	168.96±3.43	164.17±4.04	159.92±4.27	0.101
7-14	409.54±9.76	428.46±1.25	408.67±33.55	412.08±4.69	0.535
14-21	598.83±59.00	665.46±31.68	576.67±122.92	604.50±48.77	0.557
21-28	909.58±86.24	918.25±73.40	873.17±152.13	919.92±71.38	0.941
28-35	1013.42±150.51	1090.92±53.13	1070.83±11.27	1086.67±26.02	0.610
0-21	1176.71±70.82	1262.88±30.65	1149.50±155.32	1176.50±49.35	0.513
21-35	1923.00±236.66	2009.17±95.99	1944.00±153.25	2006.58±96.90	0.854
0-35	3099.71±250.80	3272.04±125.94	3093.50±302.01	3183.08±136.74	0.711

Data are Mean ± Standard deviation

Table 6. FCR of chicks fed different diets replaced with autolyzed *S. cerevisiae*

Age (days)	Level of replacement				P-value
	0%	25%	50%	100%	
0-7	1.44±0.08	1.45±0.09	1.65±0.18	1.65±0.24	0.0867
7-14	1.90±0.07	2.11±0.34	2.82±0.81	2.68±0.30	0.151
14-21	1.94±0.33	2.42±0.84	2.05±0.15	2.20±0.44	0.676
21-28	2.42±0.17	2.38±0.30	2.43±0.25	2.86±0.62	0.386
28-35	2.04±0.21	1.87±0.13	1.92±0.23	2.03±0.24	0.760
0-21	1.83±0.19	2.11±0.48	2.16±0.12	2.23±0.32	0.379
21-35	2.20±0.07	2.07±0.20	2.12±0.20	2.31±0.12	0.416
0-35	2.12±0.17	2.08±0.29	2.13±0.15	2.28±0.19	0.653

Data are Mean ± Standard deviation

Body weight gain

Body weight gain (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae* are shown in **Table 4**. The effects of replacing protein concentrate with autolyzed *S. cerevisiae* was significant during 0-7, 7-14, and 0-21 days of age. In contrary, the effect was non-significant in 14-21, 21-28, 28-35, 21-35 and 0-35 days of age. During 0-7 days of rearing, no significant difference in body weight gain was observed in 25% and 50% replacement group as compared to control group (0% replacement), whereas significantly ($P<0.05$) lower body weight gain was observed in 100% replacement group as compared to control group.

Feed intake and FCR

Table 5 represents feed intake (g) of chicks fed different diets replaced with autolyzed *S. cerevisiae*. Irrespective of stages of growth, the

effect of replacing protein concentrates with autolyzed *S. cerevisiae* had no effects on feed intake. However, numerically higher but statistically insignificant feed intake was observed in 25% and 100% replacement group as compared to control group, whereas 50% replacement group showed the lower feed intake.

FCR of chicks fed different diets replaced with autolyzed *S. cerevisiae* is represented in **Table 6**. The effect of replacing protein concentrate with autolyzed *S. cerevisiae* was insignificant throughout the trial period. However, statistically insignificant but numerically lower FCR was observed in 25% replacement group whereas 100% replacement group showed the higher.

Carcass characteristics

Carcass characteristics and organ weights of broilers fed different diets replaced with autolyzed *S. Cerevisiae* are shown in **Table 7**.

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Table 7. Carcass characteristics of broilers fed different diets replaced with autolyzed *S. cerevisiae*

Parameters	Level of replacement				P-value
	0%	25%	50%	100%	
Dressing percentage (%)	66.66±2.99	66.67±2.57	65.83±1.39	66.12±1.17	0.951
Blood wt (%)	3.10±1.10	3.60±1.25	4.13±0.83	3.97±0.26	0.568
Feather wt (%)	3.38±0.28	4.80±0.21***	3.50±0.07 ^{NS}	3.72±0.15 ^{NS}	6.61×10 ⁻⁵
Shank wt (%)	4.24±0.45	4.16±0.23	4.61±0.38	4.58±0.22	0.308
Head wt (%)	3.17±0.08	3.40±0.07 ^{NS}	3.11±0.08 ^{NS}	2.78±0.20*	0.00156
Neck wt (%)	1.97±0.41	1.79±0.15	2.68±0.30	2.36±0.39	0.0421
Abdominal fat (%)	1.04±0.23	0.98±0.09	1.07±0.11	0.72±0.08	0.0537
Liver wt (%)	2.42±0.24	2.27±0.19	2.20±0.11	2.23±0.12	0.467
Gizzard wt (%)	2.21±0.16	1.91±0.26	2.30±0.06	2.17±0.22	0.161
Spleen wt (%)	0.09±0.03	0.12±0.03	0.13±0.03	0.10±0.01	0.443
Heart wt (%)	0.56±0.05	0.59±0.15	0.62±0.15	0.54±0.07	0.844
Intestine wt (%)	8.83±2.06	8.40±0.83	8.09±1.60	8.83±0.76	0.899

Data are Mean ± SD. ^{NS}: non-significant, ***: $P < 0.001$ compared to control diet (0% replacement) as determined by Dunnett's test

The effect of replacing protein concentrate with autolyzed *S. cerevisiae* was insignificant in different carcass characteristics parameter among the dietary groups except feather and head weight. Significantly ($P < 0.001$) higher feather (%) was observed in 25% replacement group. 100% replacement group resulted significantly ($P < 0.05$) lower head weight as compared to control group.

Discussion

Body weight and body weight gain

Autolyzed *S. cerevisiae* was used to replace commercial protein concentrate in broiler ration. Partial replacement of protein concentrate did not significantly affect body weight, body weight gain, feed intake, FCR and carcass characteristics. *S. cerevisiae* is rich in protein, balanced amino acids, B-complex vitamins and minerals (Rodríguez *et al.*, 2011; Amata, 2013). This might be the cause of insignificant effect of replacing protein concentrate with *S. cerevisiae* on body weight and body weight gain. The results of this study are in agreement to findings of Owens and McCracken (2007), Morales-López *et al.*, (2009) and Brümmer (2010) who found no adverse effect of using yeast and yeast product on body weight and body weight gain in broilers. Contrary to this study, Chand *et al.* (2014) reported significantly ($P < 0.05$) higher body weight gain in broilers of different dietary

treatments replacing soybean meal with *S. cerevisiae* as compared to control feed, although *S. cerevisiae* and soybean have more or less similar essential amino acid composition (Adedayo *et al.*, 2011).

Feed intake and FCR

Insignificant differences in feed intake were observed among different dietary groups. The results of this study are in line to Chand *et al.* (2014), who have reported insignificant effect of replacing soybean meal with *S. cerevisiae* in broiler diet. Gao *et al.* (2008) have reported no effect on feed intake in broilers supplemented with 2.5, 5 and 7.5g/Kg yeast. Similar effect was reported by Hassanein and Soliman (2010). Contrary to this study, Chen *et al.* (2009) and Sharif *et al.* (2012) have reported higher feed intake in broiler chicks as a result of supplementing yeast in the ration; whereas, Abou El-Naga (2012) have reported a significant decrease in feed intake as a result of using yeast in the ration. The positive effect of using *S. cerevisiae* may be due to the unidentified growth factors along with other nutrients which are available in cells (Gao *et al.*, 2008).

Therefore, it may be postulated that replacement of commercial protein concentrate with autolyzed *S. cerevisiae* did not affect the taste of the feed and thereby feed intake. Numerically lower FCR was observed in 25% and 50% replacement groups

as compared to control group. Chen *et al.* (2009) reported that presence of unidentified growth factors in yeast cells which act on intestinal villus and improve the gross energy absorption from feed. Moreover, yeast single cell protein has higher biological value (Sharif *et al.*, 2012). These may be responsible for improved FCR in replacement groups. The findings of this study are consistent with the finding of Abou El- Naga (2012) and Yalçin *et al.* (2013). They found that addition of *S. cerevisiae* results improved FCR in broiler. In contrary to this study, Owens and McCracken (2007) reported no improvement of FCR as a result of using yeast and yeast products in broiler ration.

Carcass characteristics

No significant differences in dressing percentage, abdominal fat, gizzard, liver, heart, spleen and intestine among different dietary groups were observed in this study. The result of this study are in agreement with findings of Ozsoy and Yalçin (2011), Yalçin *et al.* (2013) and Chand *et al.* (2014) who have reported no effect of using yeast autolysate in feed on liver, gizzard, heart and dressing percentage in broilers. Contrary to this study, Onifade *et al.* (1998) reported that weight of liver, gizzard, heart and dressing percentage were significantly increased due to supplementation of yeast extracts. The result may vary due to different factors like form of feed, environment, quality of yeast protein, physiological functioning of different organs.

Conclusion

The findings of this study suggest that autolyzed *S. cerevisiae* might be used to replace protein concentrate at a level of 25% and 50% in broiler diet without compromising growth and carcass quality. However, further study with female chicks might help to recommend more precise inclusion level of autolyzed *S. cerevisiae* as a source of SCP in the broiler diet.

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Conflict of interest

We, the affiliated authors whose names are reported in the manuscript, hereby, declare that, we have NO affiliations with or involvement in any organization or entity with any financial

interest or non-financial interests in the subject matter or materials discussed in this manuscript.

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