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ORIGINAL RESEARCH ARTICLE

Effect of Different Levels of Supplemental Probiotics (*Saccharomyces cerevisiae*) on Performance, Haematology, Biochemistry, Microbiology, Histopathology, Storage Stability and Carcass Yield of Broiler Chicken

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ABSTRACT

Problem statement: As the intestinal function is intimately affected by fed diets, many kinds of natural substances, prebiotics and probiotics have been supplemented to broilers to increase poultry production by activating intestinal function. The aim of this study was to investigate whether *Saccharomyces cerevisiae* could improve growth performance, haematology, biochemistry, microbiology, storage stability and intestinal histological alterations would be observed in these birds.

Approach: A total of 200 broiler chicks were randomly assigned to 4 treatment groups, consisting of 2 replicates of 25 birds each. Commercial mash starter and finisher diets were supplemented with 0.5%, 1%, 1.5% of *Saccharomyces cerevisiae.*

Results: Body weight gain was better in all the experimental groups than the control. The growth performance was increased in 1.5% of *Saccharomyces cerevisiae.* Total cholesterol, triglyceriod was decreased, HDL, Serum Glutamine Pyruvic Transaminase (SGPT), Serum Glutamine Oxaloacetate Transaminase (SGOT), total Protein, Albumin, Globulin were increased, Total count, haemoglobin, RBC, PCV were increased at 1.5% of *Saccharomyces cerevisiae.* Lactobacillus, Yeast count were increased and Villiae length also increased at 1.5% of *Saccharomyces cerevisiae*. TBA value was decreased and carcass weight was increased in birds fed with 1.5% of *Saccharomyces cerevisiae*.

Conclusion: The present results the inclusion of 1.5% of probiotic (*Saccharomyces cerevisiae*) could improve the performance, blood constituents, histology, micro biota of intestine, storage stability and carcass characteristic of broiler chicks.

Keywords: Probiotic, Chicken, Performance and Histology.

1. INTRODUCTION

The use of antibiotics as growth promoters was completely banned in 1999 by the European Union (EU) [1]. This was due to increases in microbial resistance to antibiotics and residues in chicken meat products which might be harmful to consumers. Currently, in many parts of the world, feed additives, such as probiotics, prebiotics, are being experimented to alleviate the problems associated with the withdrawal of antibiotics from feed. Probiotic is defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [2]. Probiotics are biological products, which stimulate the immunity system and increase its defensive activity against pathogenic bacteria. Probiotics competitively exclude the Salmonella bacteria from the intestinal tract of the treated chickens. The auspicious effect of probiotics over the organism is due to the better adhesion of the lactic acid bacteria to the intestinal epithelium in comparison to the pathogenic bacteria, and stopping the implementation of those bacteria over the mucosa of the intestine [3].

Saccharomyces cerevisiae (SC), one of the most widely commercialized types of yeast, has long been fed to animals. Results of earlier studies with yeast fed to chickens, however, have not been consistent. It has been reported [4, 5, 6] that feeding yeast to chicks improves BW gain and feed/gain ratio. The bacterial populations in the gut of birds were altered when MOS were added to their diets [7-10].

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Functions of supplemental dietary microbial products in the digestive system are 1) they provide nutrient, 2) they aid digesting foods, and 3) they inhibit harmful bacteria in the gut [11]. Gastrointestinal normal flora plays an important role in the health and performance of poultry [12]. One such alternative is the addition of yeast and yeast products to poultry diets. The inclusion of nonpathogenic yeast, *Saccharomyces cerevisiae*, in the diet has been shown to improve bird performance and decrease mortality [13].

2. MATERIALS AND METHODS

2.1. Birds, diet and Experimental Period

Two hundred day-old male broiler chicks (Ross) assigned to 25 chicks of 2 treatment groups, randomly. The experimental design was completely random, consisting of three dietary levels (0.5%, 1% and 1.5%) of each two forms (powdery and granular) of *Saccharomyces cerevisiae* and a control group (without yeast) were formulated (Table 1). Each treatment had two replicates of 25 birds. Chicks fed three basal of Maize-soybean diets during three periods of 0-10 days birds fed with broiler Pre-starter, 11-20 birds fed with broiler Starter I, 21–30 days birds fed with broiler Starter II, 31-36 days birds fed with broiler Finisher. The diets supplemented with amino-acids, minerals, and vitamins to meet all the Ross requirements. The live yeast *Saccharomyces cerevisiae* (containing 1×10^9 CFU/g) was provided from Pucheng (China).

2.2. Body weight and Feed Intake Measurement:

Birds were group weighed by cage at 1, 10, 20, 30 and 36 d of age. Feed intake was monitored by cage at 10, 20, 30 and 36 d of age. Cage was the experimental unit for performance was used to calculate feed/gain ratios.

Table 1: (Pre- Broiler Starter) 1 - 10 Days feed composition under different ratios

Ingredients	Control	(0.5%)	(1%)	(1.5%)
Maize(Kg)	1.1	1.1	1.1	1.1
Soya 48% (Kg)	8.87	8.87	8.87	8.87
Crushed fish 45% (Kg)	1.5	1.5	1.5	1.5
MBM (Kg)	1	1	1	1
Ricebran oil (Kg)	0.9	0.9	0.9	0.9
Dicalcium Phosphate (Kg)	0.0125	0.0125	0.0125	0.0125
Methionine DCM (Kg)	0.0725	0.0725	0.0725	0.0725
Lysine (Kg)	0.0225	0.0225	0.0225	0.0225
Threonine (Kg)	0.0025	0.0025	0.0025	0.0025
Sodium bi carbonate (Kg)	0.0025	0.0025	0.0025	0.0025
Salt (Kg)	0.0175	0.0175	0.0175	0.0175
Choline chloride (Kg)	0.0375	0.0375	0.0375	0.0375
Additives (Kg)	0.49	0.49	0.49	0.49
Sacchromysis (Kg)	nil	0.0125	0.0250	0.0375
Total (Kg)	25	25	25	25

Table 2: (Broiler Starter I) 11th - 20th Days feed composition under different ratios

Ingredients	Control	(0.5%)	(1%)	(1.5%)
Maize(Kg)	27.92	27.92	27.92	27.92
Hypo Soya (Kg)	14.76	14.76	14.76	14.76
Crushed fish 45% (Kg)	3	3	3	3
MBM (Kg)	2	2	2	2
Ricebran oil (Kg)	1.45	1.45	1.45	1.45
Dicalcium Phosphate (Kg)	0.2	0.2	0.2	0.2
Methionine (Kg)	0.13	0.13	0.13	0.13
Sodium carbonate (Kg)	0.1	0.1	0.1	0.1
Choline chloride (Kg)	0.087	0.087	0.087	0.087
Lysine (Kg)	0.05	0.05	0.05	0.05
Salt (Kg)	0.045	0.045	0.045	0.045
Threonine (Kg)	0.015	0.015	0.015	0.015
Trace Mineral (Kg)	0.005	0.005	0.005	0.005
Toxin Binder (Kg)	0.5	0.5	0.5	0.5
Sodium Sulphate (Kg)	0.0015	0.0015	0.0015	0.0015
Copper sulphate (Kg)	0.00015	0.00015	0.00015	0.00015
H2O2	0.003	0.003	0.003	0.003
Additives(Kg)	0.15	0.15	0.15	0.15
Saccharomysis (Kg)	Nil	0.025	0.050	0.075
Total (Kg)	50	50	50	50

Table 3: (Broiler Starter II) 21st - 30th Days feed composition under different ratios

Ingredients	Control	(0.5%)	(1%)	(1.5%)
Maize(Kg)	41	41	41	41
Hypo Soya (Kg)	17.7	17.7	17.7	17.7
Crushed fish 45% (Kg)	3.4	3.4	3.4	3.4
MBM (Kg)	3.12	3.12	3.12	3.12
Ricebran oil (Kg)	2.70	2.70	2.70	2.70
Dicalcium Phosphate (Kg)	0.138	0.138	0.138	0.138
Methionine (Kg)	0.16	0.16	0.16	0.16
Sodium carbonate (Kg)	0.138	0.138	0.138	0.138
Choline chloride (Kg)	0.121	0.121	0.121	0.121
Lysine (Kg)	0.076	0.076	0.076	0.076
Salt (Kg)	0.055	0.055	0.055	0.055
Threonine (Kg)	0.020	0.020	0.020	0.020
Trace Mineral (Kg)	0.069	0.069	0.069	0.069
Toxin Binder (Kg)	0.069	0.069	0.069	0.069
Stabclo A	0.033	0.033	0.033	0.033
Stabclo B	0.033	0.033	0.033	0.033
Water	0.280	0.280	0.280	0.280
Additives(Kg)	0.208	0.208	0.208	0.208
Saccharomysis (Kg)	Nil	0.0347	0.0694	0.104
Total (Kg)	70	70	70	70

Table 4: (Broiler Finisher) 31st - 36th Days feed composition under different ratios

Ingredients	Control	(0.5%)	(1%)	(1.5%)
Maize(Kg)	57.6	57.6	57.6	57.6
Ricebran oil (Kg)	5.9	5.9	5.9	5.9
Soya 48% (Kg)	28.5	28.5	28.5	28.5
MBM (Kg)	5	5	5	5
Crushed fish 45% (Kg)	10	10	10	10
Methionine (Kg)	0.26	0.26	0.26	0.26
Lysine (Kg)	0.12	0.12	0.12	0.12
Threonine (Kg)	0.031	0.031	0.031	0.031
Sodium carbonate (Kg)	0.2	0.2	0.2	0.2
Salt (Kg)	0.19	0.19	0.19	0.19
Choline Chloride (Kg)	0.175	0.175	0.175	0.175
Trace Mineral (Kg)	0.1	0.1	0.1	0.1
Toxin Binder (Kg)	0.1	0.1	0.1	0.1
Water	0.404	0.404	0.404	0.404
Stabclo A	0.048	0.048	0.048	0.048
Stabclo B	0.048	0.048	0.048	0.048
Additives(Kg)	0.3	0.3	0.3	0.3
Saccharomysis (Kg)	Nil	0.05	0.1	0.15
Total (Kg)	100	100	100	100

Table 5: Feed analysis on 1st day of composition

Feed Analysis	control	(0.5%)	(1%)	(1.5%)
Moisture	10.01	10.45	10.5	9.76
crude protein	21.46	23.45	23.89	23
Ether extract	4.24	4.41	4.84	4.43
Crude fibre	2.58	2.76	2.76	2.81
Total ash	6.7	6.4	7.3	6.9
Sand and silica	1.2	1.4	1.3	1.3
Calcium	0.94	0.89	0.98	0.99
Total phosphorous	0.72	0.57	0.64	0.64
Salt	0.51	0.55	0.55	0.55

Data were collected for body weight gain, feed intake and feed conversion ratio during periods at 10, 20, 30 and 36 d of age.

2.3. Haematology and Biochemical analysis:

At the period of (day 36), 6 broilers were randomly selected from each replicate of each treatment group and blood samples were collected from the bronchial vein during slaughter. The collected blood samples were centrifuged at 2000 rpm for 10 min and the sera were decanted into aseptically treated vials and stored at –20 ºC until further analysis of Haematology parameter (Total platelet count, RBC, Haemoglobin, PCV, Heterrophil, Lymphocyte, Esonophil, Monophil, and Basophil) and Biochemical values (Total cholesterol, Triglyceroids, HDL, SGOT, SGPT, Total protein, Albumin and Globulin) with commercial kit (Merck,Bangalore).

2.4. Intestinal Microbiology:

Birds were killed by cervical dislocation while feeding normally. The abdominal cavity was opened, and all digest contents of ileum and cecum were immediately collected under aseptic conditions into sterile glass bags and put on ice, until they were transported to the laboratory for enumeration of microbial populations. MRS agar (MERCK, 1.10660) was used for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) was used for yeast, as the incubation medium. LAB and yeast counts of the ileum or cecum contents were obtained at 30°C degrees following 3 days incubation period. *E. coli* was grown on VRB agar (MERCK, 1.01406) aerobically at 37°C for 24-48 hours. The bacterial colonies were enumerated, and the average number of live bacteria was calculated based on per gram of original ileal and cecal contents. All quantitative data were converted into logarithmic colony forming units (cfu/g). Koc *et al* [14].

2.5. Tissue Sampling and Measurement of villus height:

The samples of the whole intestinal tract were removed, and segments of approximately 2 cm were taken from the crop near the esophageal junction, the midpoint of proventriculus, the midpoint of duodenum (duodenum), the midpoint between the bile duct entry and Meckel's diverticulum (jejunum), proximal cecum, and rectum. Segments were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. All histological studies were performed on 5-_m sections, stained by haematoxylin and eosin, and examined by light microscope. The tissue morphology was graded, and the severity of lesions was scored [15]. In the jejunum (5 sections for each segment per bird), the villus length was measured from the villus tip to the bottom.

2.6. TBA- Thiobarbituric Acid Reactive substance:

TBA value of Muscle and liver was determine according to the method describes. Muscle and liver samples that had been stored at−20°C were thawed at 4°C and homogenized. Four subsamples, weighing approximately 2.5 g, from each of the Muscle and liver samples were weighed into 50-mL screw-capped centrifuge tubes and then incubated at 30°C for 10 d. After incubation, each subsample was immediately subjected to a malondialdehyde acid (MDA) assay to measure the extent of lipid oxidation. MDA, a secondary oxidation product, was determined [16]. The amounts of 2-TBA-reactive substances (TBARS) were expressed as milligrams of MDA per kilogram of sample. The measurement of oxidative stability in skin samples was the same as outlined for Liver and Muscle samples except for the homogenization step. Intact skin samples were incubated from 0 to 10 d. immediately after incubation; skin samples were homogenized with 6 mL of 20% trichloroacetic acid and further processed as described above to measure the TBARS values.

2.7. Carcass characteristics:

At the end of the experimental period, 6 chicks from each treatment were randomly selected weighed, slaughter and dressed to determine the carcass weight and liver, gizzard, breast muscle, fat.

3. RESULTS AND DISCUSSION

3.1. Growth performance:

(Table 6) presents average value of body weight gain, feed intake and feed conversion ratio of broiler chicks fed different levels of yeast at 36 days of age. Results showed that chicks fed 1.5% yeast had the higher body weight gain and improved feed conversion ratio compared with the control group or other dietary treatments. Meanwhile, chicks fed 1.5% *S. cerevisiae* had higher feed consumption compared with the other dietary treatments (control, 0.5 and 1% *SC).* Results of the present study showed that the

inclusion of 1.5% *S. cerevisiae* yeast in broilers ration improved body weight gain, feed intake and feed conversion ratio. The obtained results confirmed the previous findings of several researchers [17, 18]. Also in agreement with our study, Onifade *et al*. [19] reported that SC improved feed/gain ratio and BW gain. These results suggest that yeast increased these parameters at an optimum level and its effect will reduce exceed of this optimum level that probably refer to digestive tract activity. It seems that the feed digestion will alter by adding more yeast and the bird growth will alter too.

Table 6 a: Effect of different ratios of *Saccharomysis cerevisae* on body weight

Day	Ctrl	0.50%	1%	1.50%
10th day	202	259	216	267
20th day	523	646	525	643
30th day	1268	1366	1181	1704
36th day	1627	1911	1951	2043

Table 6 b: Feed intake

Day	Ctrl	0.50%	1%	1.50%
10th day	169	144	167	155
20th day	748	560	562	528
30th day	1834	1565	1738	1687
36th day	2632	2549	2662	2455

Table 6 c: Feed composition ratio

Day	ctrl	0.50%	1%	1.50%
10th day	0.83	0.55	0.77	0.58
20th day	1.46	0.86	1.07	0.82
30th day	1.44	1.14	1.47	0.99
36th day	1.61	1.33	1.36	1.2

3.2. Blood constituents:

The results of plasma total protein, total plasma cholesterol, albumin, globulin, HDL, triglycerides, total SGOT, SGPT and heterophil to lymphocytes are in (Table 7). The present results showed that chicks fed 1.5% yeast had the higher total plasma protein values compared with the other dietary treatments. On the other hand, chicks fed 1.5% yeast recorded the higher albumen and globulin concentration compared with all other dietary treatments. The present results showed that feeding broiler chicks 1.5% *S. cerevisiae* reduce plasma cholesterol and triglycerides compared with broiler chicks fed control, 0.5 and 1% *S. cerevisiae*. Chicks fed ration containing 1.5% *S. cerevisiae* recorded increase the high density lipoproteins. 1.5 *S. cerevisiae* yeast significantly increased Total count and decreased hetrophil to lymphocytes ratio of chicks. Our observations corroborated data published by some authors [20, 21, 22] who stated that there was a decrease in plasma cholesterol for chicks fed diets contains yeast and different probiotics**.** Probiotics could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids. Since, the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids [23].

Table 7 a: Biochemical value of Broiler Chicken fed with different ratios of *Saccharomysis cerevisae on* 36th day

Parameters	control	(0.5%)	(1%)	(1.5%)
Total chloesterol(md/dL)	168	142	158	123
Triglyceroids(md/dL)	121	132	149	117
HDL(md/dL)	50.45	56.98	57.56	58.36
SGOT(IU/L)	122	131	144	159
SGPT(IU/L)	7	11	13	1.9
Protein(g/dL)	16	5	4.7	5.2
Albumin(g/dL)	2.1	2.9	2.9	3.3
Globulin(g/dL)	2.3	2.4	2.5	3.1

Table 7 b: Haematology value of Broiler Chicken fed with different ratios of *Saccharomysis cerevisae* on 36th day

Parameters	control	(0.5%)	(1%)	(1.5%)
Total count(cells/cumm)	4600	6800	12,300	15800
Total Platelet count(cells/cumm)	150000	142,000	168,000	172000
Haemoglobin (gm)	9	9	9.6	10.8
Haemoglobin (%)	58%	60%	64%	72%
RBC	3.1	3.1	3.4	3.6
Packed cell volume (%)	34	35	39	39
Heterophil(%)	20	20	24	39
Lympocyte(%)	54	58	76	79
Esonophil(%)	1	0	1	3
Monophil(%)	0	0	0	0
Basophil(%)	0	0	0	0

3.3. Intestinal flora:

The treatments on ileal microbiota (log cfu/g ileal content) are shown in (Table 8). In ileal digesta, LAB counts were increased and *E. coli* numbers were decreased compared to control groups Table 8b show the cecal microbiota (log cfu/g cecal content). In cecal digesta, LAB counts were significantly increased for the birds fed with 1.5% *Saccharomyces cerevisiae*, whereas *E. coli* were significantly decreased compared to control groups ($P<0.001$). An increase in the population of yeast in ileal and cecal digesta were observed for *Saccharomyces cerevisiae1.5%*. Savage and Zakrzewska [24] reported that the removal of potential pathogens from the intestinal tract of growing animals may provide a more favorable environment for the digestion, absorption, and metabolism of growth-enhancing nutrients.

Table 8 a: Effect of dietary *Saccharomysis cerevisae* on ileum microiota (cfu/g)

Treatments	control	Sac (0.5%)	Sac (1%)	Sac (1.5%)
Lactobacillus	4.9	8.2	10.5	11.9
Yeast	3.9	4.79	6.93	7.12
E.coli	3.52	2.6	2.45	2.1

Table 8 b: Effect of dietary *Saccharomysis cerevisae* on Caecum microiota (cfu/mg)

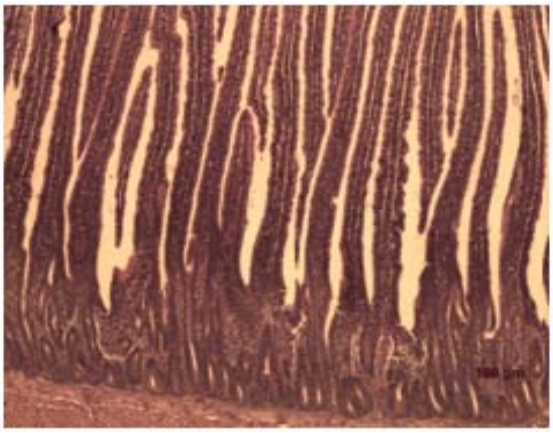
Treatments	control	Sac (0.5%)	Sac (1%)	Sac (1.5%)
Lactobacillus	4.68	7.65	8.12	10.21
Yeast	4.29	8.63	10.15	12.31
E.coli	7.6	5.13	4.96	3.8

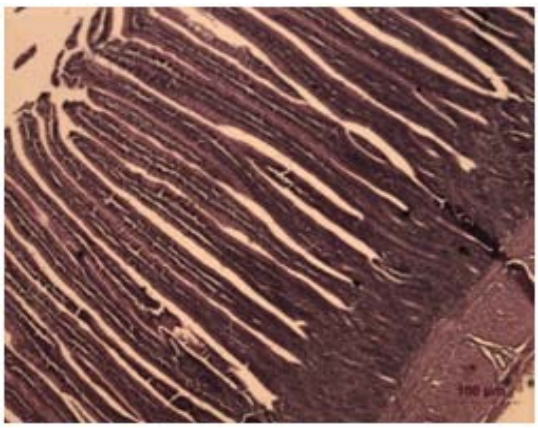
3.4. Intestinal Morphology

Villiae length was increased in fed with 1.5% *saccharomysis cerevisae* compare with control and other group was shown in (Table 9). These results are same from results previously reported and described higher villi in the intestinal mucosa of birds fed diets with MOS. This result was agreeing pelicano *et al*. [25] at 7 and 21 days of age, respectively.

Table 9: Effect of villus height on broiler chicks fed with *saccharomysis cerevisiae*

S. No	Control	Sac 0.5%	Sac 1%	Sac 1.5%
Average	207.77	276.87	279.82	326.31





3.5. Storage stability:

TBA value was significantly increased at 1.5 % of *saccharomysis cerevisae* in liver and muscle shown in (Table 10). The results provide evidence that supplementation of *SC* to a Maize-soybean meal base control diet could improve oxidative stability of broiler meat. It may indicate that there are some antioxidant factors present in *SC* or that *SC* supplementation may shift the oxidative fat (or fatty acids) profile in the meat. Some antioxidant factors in *SC* have been reported, such as glucose tolerance factor fractions (acts as an antioxidant; Ampel et al., 2000 26) and copper-zinc superoxide dismutase (acts as oxidation-retarding factor; Meyer et al., 1994 27). The *SC* CW, which contains *α*-glucan, carboxymethylglucan, mannans, and some proteinous substances, has been reported to display relatively good antioxidative properties.

Table 10: Effect of *Saccharomysis cerevisae* on storage stability by thiobarbituric acid value

Treatment	Muscle	Liver
Control	1.74	0.58
saccharomysis 0.5%	1.49	0.42
saccharomysis 1%	1.44	0.32
saccharomysis 1.5%	1.09	0.22

3.6. Carcass characteristics

(Table 11) represents meat weight, breast weight, Gizzard, leg, liver and Heart weight was increased and abdominal fat was decreased on inclusion of feed containing 1.5% of *Saccharomyces cerevisiae.* This result was agree with, Kalavathy *et al*. [27] found that supplementation of *S. cerevisiae* reduces abdominal fat.

Table 11: Carcass and organ weight of broiler chicken fed with *Saccharomysis cerevisae*

Organ	control	0.5%	1%	1.5%
Total weight	1734	1965	1972	2123
Meat weight	1283	1598	1622	1673
Breast weight	570	655	629	679
Leg weight	473	413	424	435
wings weight	153	149	130	89
Gizzard	32	30	32	37
Neck weight	51	57	58	59
Liver weight	35	40	40	42
Heart weight	7	8	9	11
Fat weight	38	35	25	19

CONCLUSION

Broiler chicks fed with 1.5% probiotics (*Saccharomyces cerevisiae*) had the higher BWG, FI, total plasma protein, plasma cholesterol and triglycerides compared with the control group or other dietary treatments. Villiae length, TBA value,Ileum and Ceacum microbiota was also increased with the inclusion of 1.5% *Saccharomysis cerevisiae and* improved the carcass characteristics of broiler chicken

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