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Probiotics Improve Physiological Parameters and Meat Production in Broiler Chicks

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ABSTRACT

The present study was planned to probe the effects of a commercial yeast product as a probiotic on growth performance and quality of meat production in broiler birds. For this purpose, 140 broiler chicks (a-day old) were purchased from a local hatchery and reared as per standard housing conditions. After three days of acclimatization, chicks were divided into seven equal groups (A to E). Group A was kept control negative. Groups B1, C1, and D1 were treated with Yeast Guard Pro® @ 1, 1.5, and 2% in feed, respectively. Groups B2, C2, and D2 were first challenged with Newcastle disease virus (NDV) along with 1, 1.5 and 2% of the yeast. Group E was kept as a control and challenged with NDV. Physiological and hemato-biochemical parameters were evaluated. The data obtained were analyzed statistically. A significant increase in the feed intake was observed from 2nd to 5th week of experiment in probiotic supplemented groups as compared. A significant change in hemato-biochemical values were also observed in yeast supplemented group. It was concluded that using *Saccharomyces cerevisiae* has beneficial effects on broiler chickens' performance and body weight gain. It also has hepatoprotective and immune stimulatory effects even the birds are exposed to Newcastle disease.

Key words: Probiotics, Meat, Poultry, Broiler, Hematology, Biochemistry.

INTRODUCTION

The increased use of antibiotics as an obligatory part of poultry and livestock production has led to the development of bacterial resistance against antibiotics (Amer et al. 2020). The spread of resistant bacteria from poultry to humans increases the risk of treatment failure by antibiotics because of resistance genes transfer (Yaqoob et al. 2022). Development of antibiotic resistance and residual effects in poultry products for human utilization led to the extensive use of probiotics, prebiotics, or a combination of both as a substitute for the utilization of antibiotics in poultry (Patterson and Burkholder 2003; He et al. 2019; Yaqoob et al. 2022). Probiotics have also been defined as feed supplements consisting of useful microbes and substances that beneficially affect the intestinal microbial balance, resulting in enhanced FCR, improved body weight gain, and decreased mortality in broiler birds (Haung et al. 2004; Panda et al. 2006; El-Sawah et al. 2020; Sabry et al. 2021). Probiotic supplementation could have the following effects: 1) modification of the intestinal microbiota, 2) stimulation of the immune system, 3) reduction in inflammatory reactions, 4) prevention of pathogen colonization, 5) enhancement of growth performance, 6) alteration of the ileal digestibility and total tract apparent digestibility coefficient, and 7) decrease in ammonia and urea excretion (Jha et al. 2020).

Probiotics consist of substances and microorganisms that enhance the intestinal tract's activity and birds' performance. In other words, probiotics can be defined as living microorganisms that, when given in feed, help produce useful microbes in the intestinal tract, which are antagonistic to harmful microorganisms (Denli et al. 2003; Rahman et al. 2009; Jha et al. 2020).

According to FAO/WHO, probiotics are live micro-organisms that, when given in a suitable amount, provide beneficial effects on host health (FAO/WHO 2001). It has been reported that probiotics are microbial supplements which when administered in sufficient amounts in feed, have beneficial effects on the microbial flora of the intestine (Kabir 2009; Salarmoini and Fooladi 2011).

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Ideal probiotics in birds' gastrointestinal tract alter the microbial structures and modify mucin degradation or synthesis, affecting the gut micro flora and resulting in increased uptake of nutrients (Patterson and Burkholder 2003; Smirnov et al. 2005; Reid 2006). They also enhance the proliferation of non-pathogenic intestinal microflora, ultimately affecting the digestion of the nutrients. Probiotics can also enhance performance of broiler birds by increasing surface area for absorption through enhancement of villi length in the small intestine (Gunal et al. 2006; Panda et al. 2006; Kabir 2009).

Various factors can affect probiotics' efficiency, including environmental factors, bird's age, dose rate and route of administration, etc. (Vineetha et al. 2017).

Different strains of microorganisms are used in probiotics like *Streptococcus, Lactobacillus, Aspergillus, Saccharomyces, Bifidobacterium, and Candida* have beneficial effects on haemato-biochemical parameters, modulation of immune response of birds, growth performance of bird, Intestinal microflora modulation and pathogen inhibition (Kabir 2009; Li et al. 2022). Among these, *Saccharomyces cerevisiae* is widely used as probiotics in broiler starter and finisher ration and is also known as baker yeast. In poultry feed, yeast products have been used as natural growth promoters (Kalia et al. 2022). Different types of yeast have been included in the diet either in the form of yeast by products, commercial yeast products, or in the form of yeast fermented mash produced on farms (Kemal et al. 2001; Saied et al. 2011; Ismael et al. 2022). It has been reported that feed conversion ratio and body weight gain improved by feeding yeast (Adebiyi et al. 2012; Khan et al. 2022).

In Pakistan, these commercial products are being widely used in poultry, including Nutriplex®, Fixir®, Fusion®, Revital plus®, and Yeast Guard Pro®. The purpose of this study was the evaluation of the probiotics *Saccharomyces cerevisiae* on the performance of birds as well as protection from the disease when challenged with local strains of Newcastle disease virus. Specific objectives were to evaluate the effect of *Saccharomyces cerevisiae*® on physiological parameters, including (Feed intake, Body weight, Organ weight), and hematological and biochemical parameters.

MATERIALS AND METHODS

Experimental birds and management: A total of 160 (day old) broiler chicks were purchased from a local hatchery and maintained under standard managemental conditions for 45 days. Fresh and clean drinking water was available around the clock. Routine vaccination for the broiler chicks as per standard recommendations was done. Standard Vaccination for the broilers for Newcastle disease (ND), infectious bursal disease (IBD) and hydropericardium syndrome was done. After three days of acclimatization, birds were divided into treatment groups (20 birds/group), as detailed in Table 1. Group A was kept as negative control and E as positive control and given the basal diet. Group B1, C1, and D1 were given feed containing *Saccharomyces cerevisiae* at three different levels. For this purpose, a new commercial product Yeast Guard Pro ® containing S*accharomyces cerevisiae* was used. However, groups B2, C2, and D2, in addition to yeast supplement with 0.2 mL of field isolated Newcastle Disease Virus (NDV) strain having ELD_{50} of $10^{-5.46}$/0.1 mL of the virus on day 30 by subcutaneous route. Group E was also challenged with NDV. Briefly, the Newcastle disease virus was isolated and then used to infect the probiotic yeast (*Saccharomyces cerevisiae*) treated broiler birds. This isolated virus was first inoculated into 9-10 days old embryonated eggs for calculation of ELD_{50} by Reed and Munch method as described by Villegas and Purchase (1989).

Physical Parameters: Physical parameters including feed intake and body weight were recorded on a weekly basis. On the 15th, 30th, and 45th day of the experiment, an equal number of birds (five/group) were slaughtered humanely. From slaughtered birds, organs including liver, kidneys, proventriculus, and duodenum were collected for absolute and relative organ weight observations by following the formula:

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Hematological Parameters: Blood samples from each bird with and without anticoagulant were collected for hematological and biochemical parameters evaluation. The hematological parameters, including TEC, TLC, hemoglobin and PCV were determined. The TEC were counted following the procedure described by Natt and Herick (Gul et al. 2022). Briefly, blood was diluted 1:100 ratio with Natt and Herrick solution. Neubar's chamber was focused at 40X, and coverslip was placed on it. Along the side of the coverslip, one drop of the diluted blood was placed, and red cells were counted at 40X. Then this figure was multiplied with 10,000 to obtain the total number of erythrocytes per cubic mm of blood by using formula (TEC/µL=(Z/80×400×100×10). The same chamber and dilution were used for the counting of leukocytes. Leukocytes were counted in all 400 squares and total number was estimated by using the formula (TLC/µL=Zx100x10).

Hemoglobin was measured through Sahil's method as described by Benjamin (1978). Briefly, 0.1N HCL was filled upto the mark 2 and then 20µL blood was added. Then it was thoroughly mixed with plastic stirrer till the hemolysis of erythrocytes. After that distilled water was added drop by drop till the color of the solution matched perfectly with the standards. Then reading was recorded at the bottom of meniscus.

PCV was determined by following the method described by Benjamin (1978). Blood was taken in plain 75x1mm hematocrit capillary tubes and then sealed with

Table 1: Experimental groups and treatment details

Groups	No. of birds	Treatment
A	20	Control –ve
B1	20	*Saccharomyces cerevisiae* @ of 1% in feed
C1	20	*Saccharomyces cerevisiae* @ of 1.5% in feed
D1	20	*Saccharomyces cerevisiae* @ of 2% in feed
B2	20	NDV+1% *Saccharomyces cerevisiae* in feed
C2	20	NDV+1.5% *Saccharomyces cerevisiae* in feed
D2	20	NDV+2% *Saccharomyces cerevisiae* in feed
E	20	Control+(Challenged with NDV)

polyvinyl powder and centrifuged at 12000rpm for 5mins in a microhematocrit centrifuge machine. After that these tubes were placed on hematocrit reader scale and value (%) was recorded.

Biochemical Parameters: Total proteins (Kit Ref 997180), albumin (Kit Ref 997258), creatinine (Kit Ref 99108), urea (Kit Ref 996060) and ALT (Kit Ref 990420) were determined through commercial kits by QCA (Quimica Clinica Aplicida, S.A. Spain) following manufacturer instructions.

Statistical Analysis: The data obtained from this experiment were subjected to statistical analysis by ANOVA, and Duncan Multiple Range Test compared the group means by using a statistical software package (MSTAT-C). The significant level was 0.05 or less than that.

RESULTS

Physical Parameters: At an early age (1-3 weeks), the feed intake in all the treatment groups was similar, and statistically, a non-significant difference was recorded (Table 2). In the later stage of experiment (4-6 weeks), the feed intake of the birds provided a higher concentration of the probiotics *Saccharomyces cerevisiae* was significantly higher than all the other groups either challenged with NDV or negative control. The lowest feed intake was recorded in group E, which was kept as control positive (Table 2).

As the probiotic supplementation has a positive effect on feed intake, ultimately the body weight gain of these groups was also good. The trend was very similar to feed intake. In the early weeks of age, all the groups recorded no significant difference in body weight gain. However, at later weeks a significantly higher weight gain was observed in the groups received probiotics (B1-D1) in feed as compared to both control and challenged with NDV groups (B2-D2) (Table 3).

The Absolute Organ Weight: There was non-significant effect of probiotics and NDV on the absolute weight of liver of broiler chicks in all groups up to the fifth week of age. At the sixth week of age there was a significant increase in absolute weight of the liver of broiler chicks in groups B1, C1, and D1 compared to control group A. The non-significant effect of probiotic and NDV was observed on the absolute weight of kidneys of broiler chicks in all groups up to 5th week of age, while at 6th week of age, the absolute weight of kidneys decreased in yeast supplemented groups as compared to control group A. There was non-significant increase in absolute weight of proventriculus of broiler chicks in all groups at 5th week of age while significant increase in absolute weight of proventriculus in group B1, C1 and D1 as compared to their infected and control groups.

The absolute weight of the duodenum of groups B1 C1, and D1 was non-significantly different from their infected groups at 2nd week of age. At the 5th week of age the absolute weight was also non-significantly different from the infected and control groups. At 6th week of age absolute weight of the duodenum of groups, C2 and D2 was significantly decreased from their non-infected groups and control group A.

The Relative Weight of Organs: The non-significant effect of probiotics was observed in the relative weight of liver of broiler chicks in all groups up to 5th week of age. From 6th week of age there was a significant decrease in the relative weight of broiler chicks in groups B1 and C1 compared to control group A (Table 5).

The non-significant effect of yeast *Saccharomyces cerevisiae* is seen on the relative weight of kidneys in all the groups. However, a relatively higher weight was observed in groups D1 and D2, those fed with 2% yeast in feed as compared to the other treatment groups. A similar trend was observed in the case of the proventriculus. The effect of the probiotic was markable in the case of the small intestine. The relative weight of the duodenum was

Table 2: Feed intake (g/bird/day) of broilers throughout supplemented with *Saccharomyces cerevisiae* as a probiotic

Groups	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
A	16.9±8.98a	46.4±9.06a	75.8±9.06a	105.1±9.02bc	134.4±9.09cd	163.8±9.08de
B1	17.3±9.29a	47.3±9.26a	77.4±9.31a	107.6±9.27abc	137.6±9.28bcd	167.7±9.27cde
C1	17.7±9.64a	48.9±9.63a	80.1±9.63a	111.2±9.64abc	142.4±9.63abcd	173.6±9.59bcd
D1	18.6±10.06a	51.2±10.07a	83.7±10.12a	116.2±10.04ab	148.8±10.08ab	181.3±10.06ab
B2	17.2±9.19a	46.8±9.16a	76.5±9.19a	106.3±9.17bc	135.9±9.23cd	165.8±9.17de
C2	17.4±9.41a	47.9±9.40a	78.3±9.40a	108.8±9.40abc	139.2±9.41bcd	169.7±9.41cde
D2	18.1±9.78a	50.1±9.83a	81.9±9.84a	113.7±9.80abc	145.6±9.83abc	177.4±9.87abc
E	16.7±8.80a	45.2±8.83a	73.9±8.82a	102.6±8.85c	131.3±8.87d	159.9±8.87e

Values (Mean±SD) having similar alphabets in a column are statistically non-significant (P>0.05).

Table 3: Body weight (g) of broiler chicks on weekly basis supplemented with *Saccharomyces cerevisiae* as a probiotic

Groups	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
A	130.6±7.57a	311.3±17.24c	641.3±38.69b	1069.3±36.25d	1527.7±111.90bc	1968.7±45.31bc
B1	135.3±9.01a	336.0±9.16bc	670.6±4.16ab	1142.7±25.74a	1668.7±87.04a	2027.3±216.97ab
C1	134.0±12.0a	340.6±16.28ab	734.6±55.47a	1252.7±24.68ab	1681.7±10.408a	2224.0±57.5ab
D1	134.6±9.86a	374.6±41.10a	718.0±62.64ab	1318.0±46.130a	1686.0±26.000a	2299.0±57.90a
B2	128.0±7.21a	335.3±12.05bc	662.6±50.01ab	1121.3±61.89cd	1582.7±72.947ab	1948.0±143.4bc
C2	140.6±11.01a	337.3±10.06bc	734.6±73.79a	1195.3±57.42bc	1601.3±44.467ab	1982.7±193.67bc
D2	137.6±16.77a	364.6±22.48ab	709.3±31.64ab	1303.3±50.32a	1627.3±48.429ab	2088.7±150.87ab
E	130.0±7.21a	314.0±18.0c	649.6±36.50b	1077.3±62.78d	1458.3±72.57c	1818.7±347.71c

Values (Mean±SD) having similar alphabets in a column are statistically non-significant (P>0.05).

relatively increased in *Saccharomyces cerevisiae* supplemented groups B1, C1, and D1 as compared to the control group at 2nd week of age. At 5th week of age, the relative weight of the duodenum was non-significantly different from their infected groups. At 6th week of age, the relative weight of C2 and D2 significantly differed from their non-infected groups (Table 5).

Hematological Parameters: There was significant increase in the total erythrocyte count of broiler chicks in all *Saccharomyces cerevisiae* supplemented groups as compared to the control group up to 4th week of age. From 6th week of age, total erythrocyte count was significantly decreased in infected groups B2, C2, D2 and E as compared to probiotic supplemented groups B1, C1, and D1 (Table 6). There was a non-significant difference in total leukocyte count of broiler chicks in all groups upto 4th week of age. At 5th and 6th week of age, total leukocyte count was significantly increased in infected groups B2, C2, D2, and E compared to *Saccharomyces cerevisiae* supplemented groups B1, C1, D1 and control group A (Table 6).

Packed cell volume of broiler chicks was significantly higher in all *Saccharomyces cerevisiae* supplemented groups as compared to control group up to 5th week of age, while at 6th week of age, there was a significant decrease in PCV of broiler chicks in groups B2, C2, D2 and E as compared to non-infected groups (Table 6). There was significant increase in the Hb concentration of broiler chicks in *Saccharomyces cerevisiae* supplemented groups (B1, C1 and D1) as compared to control groups A and E up to 5th week of age. From 6th week of age, Hb concentration was significantly lower in infected groups B2, D2, and E than in control group A (Table 6).

Biochemical Parameters: There was non-significant difference in serum alanine aminotransferase concentration of broiler chicks in all groups up to 15th and 30th day of experiment. At the 42nd day of experiment,

Table 4: Absolute weight (g) of organs of broiler chicks supplemented with *Saccharomyces cerevisiae* as a probiotic

Groups	Experimental Days					
	15th	30th	42nd	15th	30th	42nd
Absolute weight of liver			Absolute weight of kidneys			
A	9.25±0.72ab	25.10±9.19a	43.67±8.68a	2.76±0.05a	8.24±1.21a	13.34±1.62a
B1	10.09±2.10ab	27.12±8.12a	45.00±8.13a	2.79±0.23a	8.25±1.59a	12.22±0.50ab
C1	10.46±1.01a	28.33±5.24a	45.42±1.92a	2.82±0.30a	8.40±0.61a	13.10±1.48a
D1	14.77±5.26a	32.72±8.98a	50.91±9.67a	2.83±0.31a	8.77±1.33a	12.85±1.08a
B2	9.50±1.41ab	22.79±0.62a	36.80±0.81a	2.80±0.24a	8.11±2.61a	11.93±0.62ab
C2	10.17±1.21a	27.62±2.99a	43.08±6.59a	2.82±0.14a	8.38±0.59a	12.87±0.57a
D2	11.52±0.31ab	31.69±11.06a	41.08±7.21a	2.82±0.32a	8.59±1.96a	12.13±1.08a
E	9.25±0.82ab	24.72±1.09a	42.43±8.34a	2.75±0.24a	8.23±1.20a	12.54±1.17a
Absolute weight of proventriculus			Absolute weight of duodenum			
A	2.42±0.09b	4.65±2.55a	6.94±1.18ab	2.95±0.25b	6.27±1.59a	7.45±2.92ab
B1	2.95±0.49ab	4.55±1.17a	7.40±0.48ab	4.86±0.48ab	5.76±0.79a	8.70±2.66ab
C1	3.46±1.15ab	4.68±0.86a	7.68±0.23ab	4.88±0.88ab	7.05±3.04a	7.13±1.26ab
D1	3.70±0.84a	5.51±0.23a	8.81±0.56a	5.77±2.29a	9.41±0.10a	11.17±2.27a
B2	2.84±0.20ab	4.31±0.71a	7.63±1.67ab	3.93±1.18ab	5.26±0.60a	7.84±1.61ab
C2	3.06±0.04ab	4.10±0.02a	6.78± 1.18ab	4.87±0.89ab	5.13±0.60a	5.81±1.23b
D2	3.19±0.19ab	4.90±0.85a	6.72±1.03ab	5.75±2.31a	7.55±4.99a	8.43±0.26ab
E	2.40±0.07b	4.08±0.50a	5.49±0.34b	2.65±0.79b	5.68±1.50a	5.67±2.22b

Values (Mean±SD) having similar alphabets in a column under specific parameter are statistically non-significant (P>0.05).

Table 5: Relative weight (%) of organs of broiler chicks supplemented with *Saccharomyces cerevisiae*

Groups	Experimental Days					
	15th	30th	42nd	15th	30th	42nd
Relative weight of liver			Relative weight of kidneys			
A	2.43±0.49a	2.18±0.03a	3.23±0.25a	0.85±0.04a	0.48±0.04a	0.59±0.10a
B1	2.35±0.48a	2.54±0.42a	2.54±0.32c	0.93±0.04a	0.59±0.05a	0.62±0.01a
C1	2.35±0.50a	2.57±0.94a	2.73±0.25c	0.77±0.27a	0.61±0.04a	0.62±0.09a
D1	2.08±0.13a	3.02±0.03a	3.79±0.38a	1.17±0.42a	2.79±2.97a	0.72±0.17a
B2	2.20±0.37a	2.35±0.61a	2.39±0.21c	0.82±0.17a	0.60±0.14a	0.59±0.11a
C2	2.04±0.23a	2.10±0.22a	2.70±0.28b	0.78±0.26a	0.55±0.02a	0.59±0.03a
D2	2.16±0.32a	2.14±0.14a	3.62±0.37a	1.02±0.18a	0.49±0.11a	0.65±0.03a
E	2.58±0.15a	2.64±0.03a	3.21±0.40b	0.71±0.08a	0.61±0.12a	0.56±0.10 a
Relative weight of proventriculus			Relative weight of duodenum			
A	0.85±0.09a	0.43±0.11a	0.37±0.05a	0.85±0.30b	0.44±0.07a	0.44±0.11ab
B1	0.87±0.08a	0.43±0.02a	0.35±0.34a	1.20±0.02ab	0.69±0.28a	0.53±0.26ab
C1	0.89±0.10a	0.42±0.03a	0.40±0.01a	1.39±0.07ab	0.73±0.12a	0.33±0.07ab
D1	0.95±0.11a	2.39±2.67a	0.46±0.10a	1.79±0.63a	2.35±2.54a	0.59±0.13a
B2	0.87±0.09a	0.33±0.05a	0.31±0.07a	1.19±0.01ab	0.46±0.07a	0.42±0.08ab
C2	0.80±0.04a	0.40±0.09a	0.35±0.06a	1.15±0.24ab	0.72±0.31a	0.27±0.04b
D2	0.90±0.09a	0.46±0.06a	0.41±0.09a	1.77±0.65a	0.49±0.07a	0.38±0.02ab
E	0.85±0.09a	0.43±0.12a	0.34±0.06a	0.86±0.07b	0.43±0.07a	0.30±0.12ab

Values (Mean±SD) having similar alphabets in a column under specific parameter are statistically non-significant (P>0.05).

Table 6: Hematological Parameters showing effects of treatments in broiler chicks supplemented with *Saccharomyces cerevisiae* as a probiotic

Groups	Experimental Days					
	15th	30th	42nd	15th	30th	42nd
Total erythrocyte count (10^3µL)				Packed cell volume (%)		
A	2.40±0.13c	2.61±0.37d	2.20±0.08c	21.5±0.70 b	24.0±1.4c	23.0±1.41bc
B1	2.64±0.16b	3.62±0.08b	2.91±0.28b	22.5±0.70 ab	26.0±0.01ab	25.0±1.00ab
C1	2.91±0.24a	3.73±0.14ab	2.93±0.04b	23.5±0.70 ab	27.0±0.01ab	26.0±1.01ab
D1	3.25±0.22b	3.87±0.06a	3.88±0.09a	26.5±1.41 a	28.0±0.00a	28.0±1.01a
B2	2.60±0.25b	3.50±0.07b	2.70±0.17b	22.5±1.41 ab	25.0±0.01bc	24.0±1.41bc
C2	2.80±0.17a	3.72±0.13ab	2.85±0.1b	23.5±0.70 ab	27.00±0.0ab	24.0±1.41bc
D2	3.20±0.19b	3.87±0.10a	3.76±0.11a	26.0±1.41a	25.5±2.12 ab	24.5±0.70bc
E	2.26±0.18c	2.51±0.20c	2.10±0.10c	20.0±1.24 ab	24.0±1.41c	20.0±2.2c
Hemoglobin (g/dL)				Total leukocytic count (10^3µL)		
A	9.5±0.14b	10.6±0.21b	11.0±0.28c	21.14±1.60a	23.51±1.18b	25.89±3.99b
B1	10.5±0.42a	10.8±0.00ab	11.1±0.07bc	22.18±2.79a	25.39±2.75b	23.76±2.39a
C1	10.8±0.07a	11.0±0.21ab	11.4±0.07b	23.01±1.96a	25.93±3.85b	23.29±1.94a
D1	11.2±0.49a	11.5±0.42a	12.4±0.14a	23.24±1.58a	26.21±3.96b	25.56±3.58a
B2	10.7±0.63a	10.7±0.14b	10.9±0.00c	22.17±2.78a	23.29±1.94a	26.22±3.95b
C2	10.9±0.56a	10.9±0.21ab	11.1±0.07bc	23.00±2.83a	24.29±2.20a	24.48±1.84b
D2	11.1±0.49a	11.5±0.56a	11.3±0.07b	23.24±1.58a	30.04±1.85a	31.03±0.59a
E	9.5±0.28b	10.5±0.35b	10.3±0.21d	21.55±2.06a	32.03±1.19a	29.36±0.79a

Values (Mean±SD) having similar alphabets in a column under specific parameter are statistically non-significant (P>0.05).

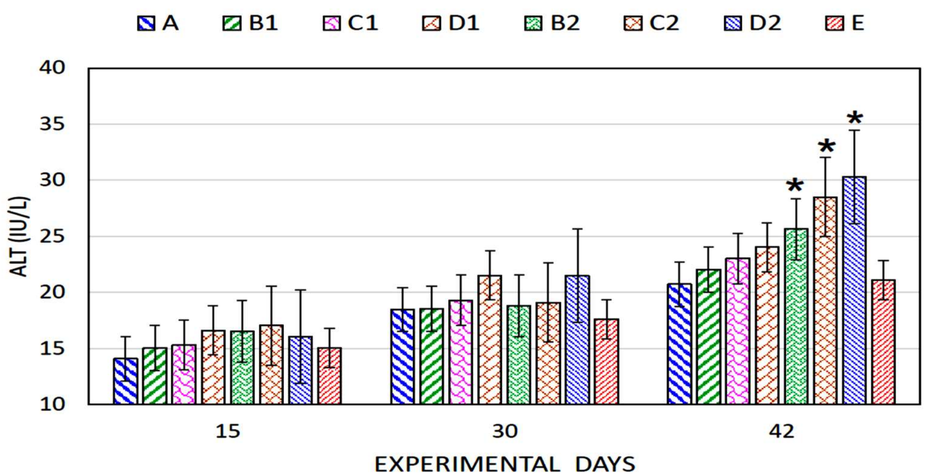

Fig. 1: Serum alanine aminotransferase in broiler chicks showing the effect of supplemented probiotics (*Saccharomyces cerevisiae*). Bars bearing asterisk differ significantly (P<0.05) than control. Group B2, C2 and D2 (challenged with NDV on 30th day) have significantly (P<0.05) higher values of ALT as compared to other experimental groups.

there was a significant increase in serum alanine aminotransferase concentration of broiler chicks in infected groups B2, C2 and C2 compared to other groups (Fig. 1). As all these groups were challenged with NDV at 30th day so this increase in ALT was observed in these groups after this challenged.

There was a non-significant difference in serum total proteins of broiler chicks in all groups up to 15th day of experiment as shown in Figure 2. At 30th day, a significant increase in the total proteins was observed in all the groups supplemented with *Saccharomyces cerevisiae* except controls (A and E). The very similar trend in total proteins values was observed on 42nd day of experiment, where the group C1, D1, B2 and C2 have significantly higher values as compared to other treated groups and controls (Fig. 2). There was a non-significant difference in serum albumin of broiler chicks in all groups throughout the study, but an increasing trend in albumin of broiler chicks was observed in all groups at 30th and 42nd day of experiments (Fig. 2). The non-significant difference was observed in serum globulin of broiler chicks in all experimental groups up to 15th day of experiment. However, a significant increase in globulin was observed in all probiotic supplemented groups at 30th and 42nd day of experiment, which is indicating the immunostimulants effects of yeast as a probiotic (Fig. 2). The globulin values for both the controls (A and E) were comparable and significantly lower than the probiotic supplemented groups suggesting the promising effects of probiotic supplementation both in healthy and disease challenged birds.

The non-significant effect of probiotic supplementation was observed on serum creatinine and blood urea nitrogen concentration of broiler chicks throughout the experiment, but an increasing trend in serum creatinine concentration was observed in probiotic supplemented groups (D1 and D2) was observed at 30th and 42nd day of experiments as compared to the control (Fig. 3).

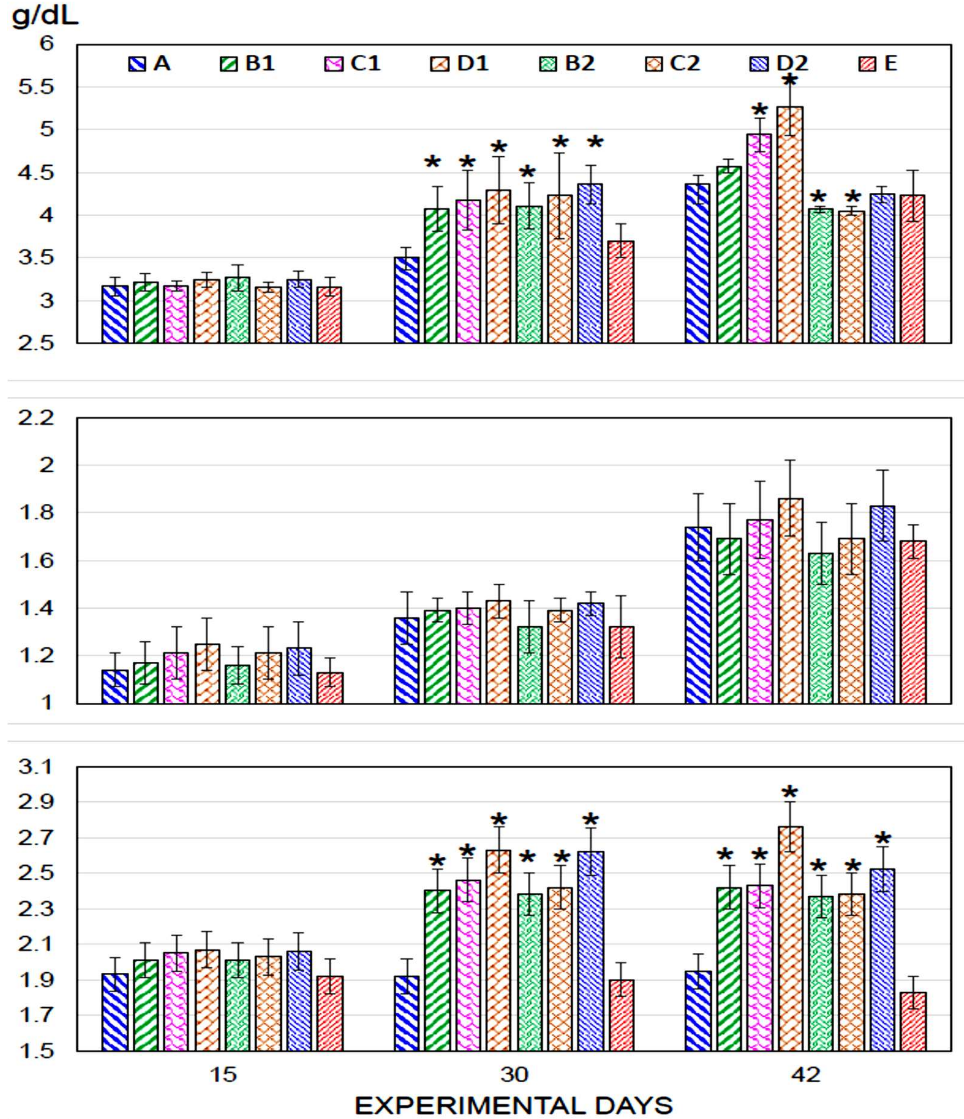

Fig. 2: Total proteins (upper), albumin (middle) and globulin (lower) in broiler chicks showing the effect of supplemented probiotics (*Saccharomyces cerevisiae*). Bars bearing asterisk differ significantly (P<0.05) than control. On 30th day group B1-D2 have significantly (P<0.05) higher Total Proteins (Upper) and Globulins (lower) values than controls. On 42nd day the trend is similar for Globulins (lower). For albumin, groups (C1-C2) have significantly (P<0.05) higher values as compared to others.

DISCUSSION

The current study was designed to investigate the effects of *Saccharomyces cerevisiae* on physiological performance and hemato-biochemical effects in broiler birds that are being used as a major source of meat production around the globe including in Pakistan. Hence the results obtained during this experimental trial are the following: At the very first week of age, a non-significant effect of probiotics on the body weight of broiler chicks in all experimental groups was recorded. From 2nd to 6th week of age, body weight was significantly higher in probiotic supplemented groups (B1, C1, and D1) compared to control groups A and E. These results are in accordance with previous studies (Zhang et al. 2005). They reported that adding probiotics @ 3g/kg in basal feed led to increased body weight and enhanced feed conversion ratio (FCR) in broiler birds. Previously Mohan et al. (1998), Jin et al. (1996; 1998), and Khaksefidi and Rahimi (2005) reported that different strains of bacteria and yeast being used as probiotics resulted in higher body weight gain in broiler birds. Hiss and Sauerwein (2003) observed an increase in daily weight gain of broiler birds by supplementing β-glucan (*Saccharomyces cerevisiae*) in basal feed and reported hepatoprotective effects. So, it has been reported that the supplementation of probiotics in the diet has positive effects on FCR and, ultimately, body weight gain in the broiler birds (Vineetha et al. 2017; He et al. 2019; Mousavi et al., 2018; Zhang et al., 2021; Ebeid et al. 2021). However, it has also been reported that the probiotics supplementation does not always have positive effects on growth parameters and that can be dependent upon the type, strain of bacteria, species, environmental factors and many other possible factors (Fathi et al. 2017; Ebeid et al. 2021).

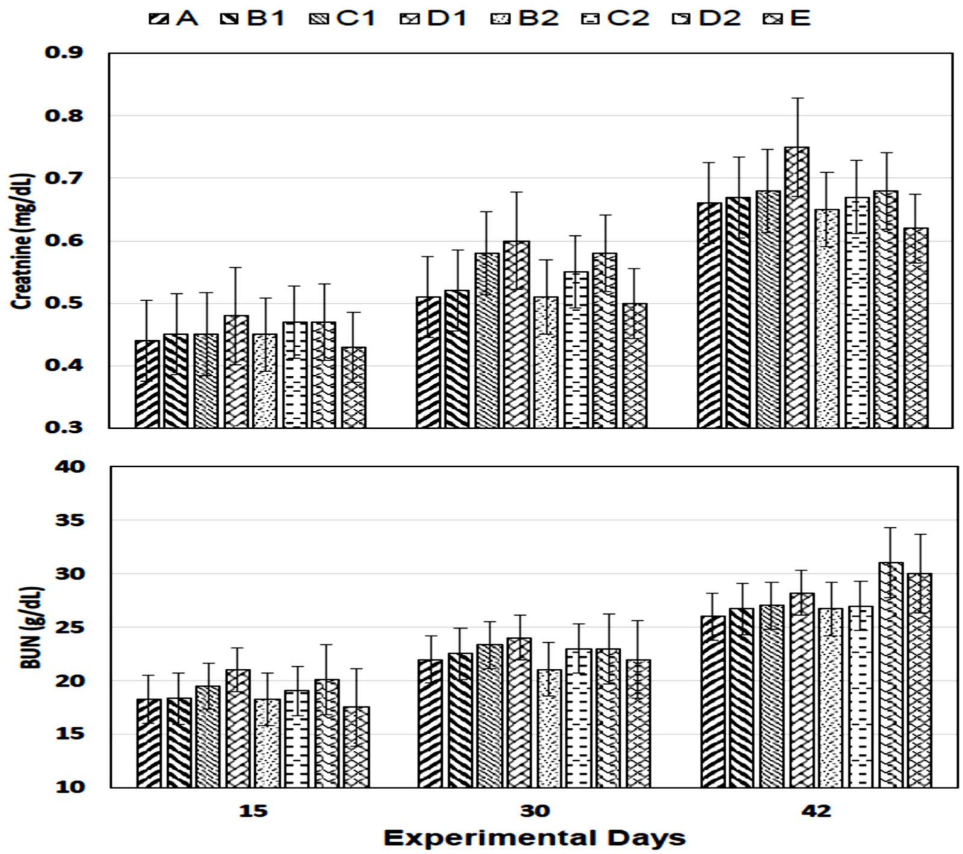

Fig. 3: Creatinine and blood urea nitrogen in broiler chicks showing the effect of supplemented probiotics (*Saccharomyces cerevisiae*). All the experimental groups (A-E) on all experimental days differ non-significantly ($P>0.05$) from each other.

In the present study, there was non-significant effect of probiotic supplementation on the absolute weight of organs (liver and kidney) of broiler chicks in all groups up to 5th week of age. At 6th week of age, there was significant increase in the absolute weight of the liver of broiler chicks in groups B1, C1, and D1 compared with control group A. Similar effect was observed in terms of the relative weight of the liver upto 5th week. However, on 6th week of age, a significant decrease in the relative weight of liver of broiler chicks in groups B1 and C1 was observed as compared to control group A. In contrast to the liver, relatively higher weight of kidneys was observed in groups D1 and D2, those fed with 2% yeast in feed at 6th week of the trial. Previously a significant increase in the liver weight has been reported (Zhang et al. 2021; Ebeid et al. 2021). However, these findings in terms of the liver, where the probiotics supplementation is linked with decrease in relative weight of liver in the current are supported by Christopher (1988), who also reported that the relative weight of the liver significantly decreased by adding *Lactobacillus* to feed. There was a non-significant increase in the absolute weight of broiler chicks' proventriculus in all groups at 5th week of age, while a significant increase in the absolute weight of proventriculus in groups B1, C1, and D1 as compared to NDV infected and control groups. The non-significant effect of probiotics was observed on the relative weight of proventriculus up to 6th week of age throughout the experiment. Previously, Jin et al. (1998) and Bozkurt et al. (2005) have also reported that there is no significant effect on the weight of proventriculus after the addition of probiotic supplementation in feed. These effects have also been supported by the Fathi et al. (2017) and Ebeid et al. (2021) that every probiotic supplementation does not have always promising effects due to various animal or related factors.

The absolute weight of the duodenum of groups B1, C1, and D1 was non-significantly different from their NDV infected groups at 2nd week of age. At 5th week of age, absolute weight was also non-significantly different from the infected and control group. At 6th week of age absolute weight of the duodenum of groups C2 and D2 was significantly decreased from their non-infected groups and control group A. Santin et al. (2001), and Zhang et al. (2005) reported that the supplementation of yeast in feed improved the growth performance and height of villus. The relative weight of the duodenum was relatively increased in *Saccharomyces cerevisiae* supplemented groups B1, C1, and D1 compared to the control group at 2nd week of age. At 5th week of age, the relative weight of the duodenum was non-significantly different from their infected groups. At 6th week of age, the relative weight of C2 and D2 significantly differed from their non-infected groups.

There was a significant increase in the total erythrocyte count of broiler chicks in all *Saccharomyces cerevisiae* supplemented groups compared to the control group up to 4th week of age, per the results reported by

Neveling et al. (2020). From 6th week of age, total erythrocyte count was significantly decreased in infected groups B2, C2, D2 and E as compared to probiotic supplemented groups B1, C1, and D1. Onifade et al. (1999), Onifade (1997), Zuhra et al. (2018) and Kumar et al. (2021) reported that adding yeast *Saccharomyces cerevisiae* in the basal feed results in higher hemogram values, including total erythrocytes, leukocytes, and packed cell volume. There was a non-significant difference in total leukocyte count of broiler chicks in all groups upto 4th week of age. At 5th and 6th week of age, total leukocyte count was significantly increased in NDV challenged groups (B2, C2, D2, and E) compared to *Saccharomyces cerevisiae* supplemented groups (B1, C1, D1, and control group A). Previously this TLC increase has been attributed to the yeast supplement in various species like rats, rabbits, and broiler (Liang et al. 1998; Fleisher et al. 2000; Seyidoglu et al. 2013; Zuhra et al. 2018). The increase is because yeast extracts have two different bioactive components: MOS and β-glucan. This β-glucan is responsible for the TLC increase in broilers (Matur et al. 2011). This TEC and TLC increase in the birds has been attributed to the immunostimulant effects of the probiotics that ultimately lead to this increase in TLC particularly lymphocytes percentages (Aathouri et al, 2001; Kumar et al. 2021)

Packed cell volume of broiler chicks was significantly higher in all *Saccharomyces cerevisiae* supplemented groups as compared to the control group upto 5th week of age. In contrast, at 6th week of age, there was significant decrease in PCV of broiler chicks in groups B2, C2, D2, and E as compared to non-infected groups. Gheisari et al. (2006) reported that supplementing yeast *Saccharomyces cerevisiae* at the rate of 0.2% in feed results in the increased value of packed cell volume, hemoglobin, and red blood cells. However, Saied *et al*. (2011) reported no significant increase in PCV value by supplementing yeast in feed. There was significant increase in the Hb concentration of broiler chicks in all *Saccharomyces cerevisiae* supplemented groups (B1, C1, and D1) compared to control groups A and E up to 5th week of age. From 6th week of age, Hb concentration was significantly lower in infected groups B2, D2, and E as compared to control group A. Seyidoglu et al. (2013) and Zuhra et al. (2018) reported that Hb concentrations were increased by supplementation of yeast, phyto-substances and probiotics in feed.

There was non-significant difference in serum alanine aminotransferase concentration of broiler chicks in all groups up to 15th and 30th day of experiment. These results are in accordance to the results reported by Abdel-Fattah et al. (2008), that supplementation of probiotics did not cause alterations in the ALT level, which proves that it is safer option for the liver, and it has been endorsed by Zuhra et al. (2018). At the 42nd day of experiment, there was a significant increase in serum alanine aminotransferase concentration of broiler chicks in infected groups B2, C2 and C2 compared to other groups and it is accordance to the results reported by Schimidt et al. (2007), This change in ALT level can be attributed the tissue damage induced by the NDV in the birds.

There was a non-significant difference in serum total proteins of broiler chicks in all groups up to 15th day of experiment. At 30th day, a significant increase in the total proteins was observed in all the groups supplemented with *Saccharomyces cerevisiae* except controls (A and E). The very similar trend in total proteins values was observed on 42nd day of experiment. There was a non-significant difference in serum albumin of broiler chicks in all groups throughout the study, but an increasing trend in albumin of broiler chicks was observed in all groups at 30th and 42nd day of experiment. Line et al. (1997) and Kumar et al. (2019) reported that supplementing yeast culture in diet results in increased serum protein and to some extent albumin levels.

A significant increase in globulin was observed in all probiotic supplemented groups at 30th and 42nd day of experiment, which is indicating the immunostimulants effects of yeast as a probiotic. The globulin values for both the controls (A and E) were comparable and significantly lower than the probiotic supplemented groups suggesting the promising effects of probiotic supplementation both in healthy and disease challenged birds. Eggum (1989) and Kumar et al. (2019) reported that supplementing probiotics in feed results in increased serum total protein especially globulin concentrations. So this enhanced production of globulins can be attributed to more immunoglobulins that provides immunity to the birds from the invading pathogens.

The non-significant effect of probiotic supplementation was observed on serum creatinine and blood urea nitrogen concentration of broiler chicks throughout the experiment, but an increasing trend in serum creatinine concentration was observed in probiotic supplemented groups (D1 and D2) was observed at 30th and 42nd day of experiments as compared to the control. Lowe and Kershaw (1997) also have reported that probiotic supplementation led to increased blood urea concentration in cats but it can be a species variation as the physiology of the renal system in mammals and avian species is quite different. Furthermore, it is stated that the exact mechanism of action for the yeasts has not been described in detail yet needs more exploration by scientific studies in terms of poultry. Only the positive effects on GIT have been reported in poultry, it has been defined that yeast supplementation in diet can increase the height of villi and reduce the depth of crypts, ultimately having positive effects on FCR and carcass percentage (Nilson et al. 2004; Gosh et al. 2011; Kalia et al. 2022).

Conclusion

It was concluded that using *Saccharomyces cerevisiae* has beneficial effects on broiler chickens' performance and body weight gain. It also has hepato-stimulatory and hepatoprotective effects that cause the improvement in physiological parameters and some hematobiochemical parameters of chicken. This *Saccharomyces cerevisiae* supplementation also proved to be effective in birds challenged with NDV being an immunostimulant. For safer and quality meat production, these probiotics can be used not as an alternative to antibiotics, but also as a growth promoter to ensure food security in developing countries.

Conflict of interest

The authors declare that they have no competing interests.

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