Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.2 / 001-008

http://dx.doi.org/10.1590/1806-9061-2021-1613

Original Article

Effects of Saccharomyces Cerevisiae Supplemented Diet on Production Performance, Egg Quality and Humoral Immunity in Black Australorp and Fayoumi Layers

■Author(s)

Sarwar F[I] https://orcid.org/0000-0003-2701-2127
Akhtar R[II] https://orcid.org/0000-0003-0501-3989
Akram Q[III] https://orcid.org/0000-0002-1084-9089
Rizwan HM[IV] https://orcid.org/0000-0002-6048-7610
Naeem MA[V] https://orcid.org/0000-0003-4366-298X
Azad A[II] https://orcid.org/0000-0003-2330-1730
Rahman MS[I] https://orcid.org/0000-0002-2259-9670
Saleem MM[VI] https://orcid.org/0000-0002-3050-7115
Anwar MN[VII] https://orcid.org/0000-0002-4762-1635
Zahid B[VIII] https://orcid.org/0000-0003-2050-9173
Tariq M[I] https://orcid.org/0000-0002-9148-9555
Habib Z[IX] https://orcid.org/0000-0003-9356-0661

[I] Poultry Research Institute, Livestock and Dairy Development Department, Rawalpindi 46300, Punjab, Pakistan
[II] Department of Pathology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan
[III] Department of Pathobiology (Microbiology Section), KBCMA College of Veterinary and Animal Sciences, Narowal 51600, Pakistan.
[IV] Department of Pathobiology (Parasitology Section), KBCMA College of Veterinary and Animal Sciences, Narowal 51600, Pakistan.
[V] Department of Basic Sciences (Pharmacology Section), University College of Veterinary and Animal Sciences, Narowal 51600, Pakistan.
[VI] Department of Animal Sciences (Poultry Production Section), University College of Veterinary and Animal Sciences, Narowal 51600, Pakistan.
[VII] Institute of Microbiology, University of Agriculture, Faisalabad 38000, Pakistan
[VIII] Department of Pathobiology (Pathology Section), KBCMA College of Veterinary and Animal Sciences, Narowal 51600, Pakistan.
[IX] Livestock Production Research Institute, Livestock and Dairy Development Department, Bahadurnagar Okara 56300, Punjab, Pakistan

■Mail Address

Corresponding author e-mail address
Raheela Akhtar
Department of Pathology, University of Veterinary and Animal Sciences, Lahore 54000, Punjab, Pakistan.
Phone: (+0092) 3036382900
Email: raheela.akhtar@uvas.edu.pk

■Keywords

Egg production, Egg Quality, Immunity, Probiotic, *Saccharomyces cerevisiae*.

Submitted: 13/December/2021
Approved: 28/September/2022

ABSTRACT

The present study intended to identify the effects of the *Saccharomyces cerevisiae* yeast on production performance and parameters related to the quality of layers' eggs. Effects of yeast on immune titers after routine vaccinations against Newcastle disease and infectious bronchitis were also studied. Fayoumi chicken (n=288) were divided in four groups (72 in each), and Black Australorp (n=288) in four groups (72 in each). Both had 12 weeks of age and were kept in 08 groups (3 replicates per treatment). Hens were fed a controlled diet along with the addition of 0.5g, 1.0 g, and 1.5 g of *S. cerevisiae* per kg of feed, till 25 weeks of age. Feed intake and egg production were measured on a daily basis, while body weight gain and egg weight were measured weekly. Egg quality parameters were evaluated by picking 5 eggs from every group weekly. Birds were vaccinated for Newcastle disease (ND) and infectious bronchitis (IB) at the 18th week. Subsequently, 10 days after vaccination, antibody titers were determined by Hemagglutination Inhibition and Enzyme Linked Immunosorbent Assay for both diseases. Results showed no significant effect of *S. cerevisiae* on layers' weight gain and feed intake. However, egg production was increased in the experimental group. Moreover, yeast supplementation impacted positively on birds' immune system. In conclusion, probiotic supplementation improved birds' egg production and immunity.

INTRODUCTION

Poultry meat and eggs are important protein sources for humans. According to Leeson (2012), there has been an increase in demand for food products, causing a qualitative and quantitative increase in production in the poultry industry. The use of probiotics and prebiotics in the poultry industry is encouraged due to the rising issue of antimicrobial resistance (AMR) and antibiotic residues' incorporation in poultry products Patterson & Burkholder (2003). According to WHO/FAO (2001), probiotics are micro-organisms that live in nature and confer benefits to the host, when given in ample amounts. Probiotics comprise live microbe cultures, such as yeast, fungi, and bacteria that positively affect the health and nutrition status of animals by developing their intestinal microbes Khan *et al*. (2011); Zhang & Kim (2014). The most widely used type of yeast, *Saccharomyces cerevisiae,* has been reported to improve feed quality and the performance of animals Martin *et al.* (1989). Major effects of using probiotics include growth improvement, reduction in mortality, Kumprecht & Zobac (1998) and better feed conversion efficiency Yeo & Kim (1997). Supplementing with *Pediococcus acidilactici* showed improvement in the weight of eggs and quality of eggshells along with reducing the percentage of broken eggs in layers Mikulski *et al.* (2012). Supplementing layer diets

with probiotics has shown evidences of reducing the concentration of cholesterol in egg yolk Abdulrahim *et al.* (1996) and serum in chicken Jin *et al.* (1998). Multi-strain probiotics have been reported to lower feed conversion ratios and numbers of damaged eggs Balevi *et al*. (2001). Immuno-modulation is also one of the benefits published in literature Salianeh *et al*. (2011). Nayebpor *et al*. (2007) and Apata (2008) reported the positive immune-modulatory effects of using probiotics. Supplementation of probiotics in the diet may have substantial effects in different species of animals and even among different breeds within species Fasina & Thanissery (2011); Fathi *et al*. (2012). Therefore, the current study was designed to investigate the effects of supplementing a *S. cerevisiae* probiotic on egg production, egg quality, and immune response on Fayoumi and Black Australorp layers.

MATERIALS AND METHODS

The study was carried out at the poultry farms of the Directorate of Poultry Research Institute, Rawalpindi, Pakistan. Egg analysis was carried out at the Feed and Water Testing Laboratory – Poultry Research Institute, Rawalpindi, Pakistan using an Automatic Egg Quality Analyzer. Afterwards, IB and ND antibody titers were assayed at the Disease and Diagnostic Laboratory, Poultry Research Institute, Rawalpindi.

Pullets

The experiment was carried out by allocating 576 pullets, 12 weeks old (Fayoumi and Black Australorp) into four groups of each strain, i.e., 288 Black Fyuomi (n=72 each) and 288 of Australorp (n=72 each). The details of group names and inclusion levels of the probiotic are given in Table 1. The experiment lasted up to 25 weeks.

Table 1 – Inclusion Level of Probiotic.

Sr. No.	Group Name	Description	Inclusion Rate
1	A1F	Probiotic (Fayoumi)	0.5 g / kg of feed
2	A2F	Probiotic (Fayoumi)	1.0 g / kg of feed
3	A3F	Probiotic (Fayoumi)	1.5 g / kg of feed
4	CF	CONTROL Fayoumi	No probiotic
5	A1B	Probiotic (Black Australorp)	0.5 g / kg of feed
6	A2B	Probiotic (Black Australorp)	1.0 g / kg of feed
7	A3B	Probiotic (Black Australorp)	1.5 g / kg of feed
8	CB	CONTROL Black Australorp	No probiotic

Bird Management

All groups were kept on floor in separate pens equipped with round feeders and automatic drinker sand. A controlled diet in crumbs form was provided during the whole experimental phase to meet the nutritional requirements of layer birds. This basal diet was supplemented by incorporating probiotic preparations in different concentrations. Ventilation, ambient temperature, humidity, lighting, litter, and other environment conditions met the management requirements of layers. No cross-contamination among groups was permitted. Birds were vaccinated against IB and ND at the 18th week of age.

Probiotic

Commercial probiotic (Actera Yeast) ® containing *S. cerevisiae* with live yeast cell count ≥ 20 billion/g was supplemented in feed at the 0.5 gm, 1.0 gm, and 1.5 gm/kg levels, and was given throughout the study time *i.e.,* up to 25 weeks. The composition of the probiotic is shown in Table 2.

Table 2 – Probiotic Composition.

Ingredient	Composition
Live Yeast Cell Count (Billion/g)	≥ 20
Protein %	≥ 46
Ash	≤ 7
Arsenic	≤ 2ppm
Lead (Pb)	≤ 10ppm
Vitamin B Complex	Positive
Salmonella	Negative

Performance Evaluation

Performance was evaluated by measuring the intake of feed and the amount of eggs produced on a daily basis. Weight gain and egg quality parameters (external and internal) were assessed weekly. Feed Conversion efficiency (FCE) was also calculated for each treatment. FCE was calculated as grams of consumed feed per gram of egg produced.

Egg Quality Evaluation

Egg production was measured daily. Afterwards, samples of eggs were weighed and egg parameters were assessed on a weekly basis. 5 eggs from each group were collected every week to assess egg quality. Parameters were egg weight, yolk weight ratio, yolk color, albumen weight, albumin ratio, albumin height, egg shape index, egg shell weight, egg shell ratio, shell thickness, and Haugh unit. Eggs were first weighed and then broken on a plane surface/egg analyzer plate and the height of the albumen was measured by using an albumen height gauge. Eggs were analyzed by an automatic egg analyzer machine and egg weight, Haugh unit, albumin weight, and yolk weight were measured. Before evaluating the yolk weight, each egg yolk was rolled on blotting paper in order to

remove the remaining albumen. Subsequently, egg albumin percentage and egg yolk percentage were calculated. Yolk color was measured by Roche's egg yolk color fan. The egg shells were left to dry out at room temperature and then weighed. Shell thickness was measured by a micrometer from top, equator, and truncated edge of egg and was averaged and recorded as shell thickness. Afterwards, the shell was left to dry at room temperature and weight was also calculated as a percentage. The maximum length and width of each egg was measured with calipers, and their shape index was determined by the formula:

Shape index = [maximum width in mm / maximum length in mm] x 100

Antibody Titer against ND virus

The Hemagglutination Inhibition (HI) test was used to determine Antibody titer against Newcastle disease virus Thayer & Beard (1998). The procedure was performed at the Disease Diagnostic Laboratory, Poultry Research Institute, Rawalpindi, Pakistan. Blood samples from 5 birds of each group were collected on the 10th day post vaccination. Separated serum was processed for the HI test.

Antibody titer against IB virus

From each group, 5 blood samples were collected 10 days after vaccination and serum was further processed. The presence of IBV antibodies were assayed using an ELISA kit (IDEXX® Laboratories Inc. United States) at Disease and Diagnostic Lab, Poultry Research Institute, Rawalpindi, by following the manufacturer's instructions.

Statistical Analysis

The data was analyzed against each parameter using one way ANOVA that compared each parameter in 3 groups with control for both Black Australorp and Fayoumi, separately. Significant parameters were represented with a "*" in superscript of its p-value and non-significant with "ns". The number of "*" represents the level of significance of the parameter. Moreover, significant parameters were further compared in group pairs using Tukey's Multiple Comparison Test (TMCT). The whole statistical analysis was performed on Graph pad Prism version 6.01.

RESULTS

Production Performance (Black Australorp)

Out of the several analyzed production performance parameters, only egg weight was found significant for Black Australorp birds. Regarding egg weight, all the three treatment groups of black Australorp showed significant increase compared to the control group (Fig. 1, Table 3). This means that *Saccharomyces cerevisiae* supplementation has a positive impact on egg weight gain. Mortality of layers in the control groups was not significantly different from layers in the experimental groups. Increasing the dose of probiotic supplement did not cause any considerable effect on mortality. Our results were in contrast with Netherwood *et al*. (1999), who reported there was an increase in survival rate (decrease in % mortality) by supplementing probiotics, due to modification in gut microflora. Likewise, another study by Ehrmann *et al*. (2002) showed suppression in growth rate of pathogenic bacteria by using probiotics. Body weights were measured weekly. Effect of *Saccharomyces cerevisiae* on production parameters through one way ANOVA and TMCT are given in Table 3.

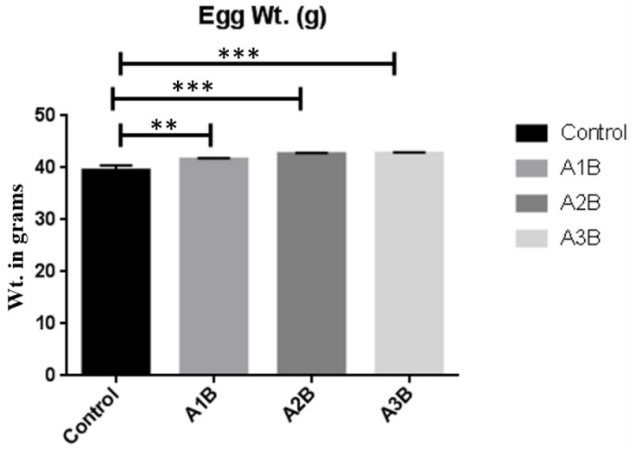

Figure 1 – Significant production parameter is shown for Black Australorp birds.

There was no significant difference in the body weights and feed intake within treatment groups fed different levels of probiotic, as shown in graph 1 and 2. The average feed intake (98g/day) was lowest in group A3B (Black australorp) 1.5 g probiotic/ kg feed, when judged against the control group (104 g/ day). Maximum egg production was observed in the Black Australorp group fed with 0.5 g probiotic/ kg of feed. Egg production was recorded as 66%, while the control groups had an average egg production of 53.3%.

Egg Quality (Black Australorp)

The traits of eggs during 20-25 weeks of age including egg weight, Haugh unit, albumin and yolk weight, albumin percentage, egg yolk percentage, albumin height, egg shape index, egg shell weight, and shell thickness were measured. The effect of

Table 3 – Effect of *Saccharomyces cerevisiae* on production parameters.

One way ANOVA				
	For Group Fayoumi		For Group Black Australorp	
Factors	Mean ± SD	*p*-value	Mean ± SD	*p*-value
Weight Gain (kg)	1.243 ± 0.096	0.5582^{ns}	1.479 ± 0.141	0.9882^{ns}
Avg. Feed Intake /day (g)	100.75 ± 3.594	0.9596^{ns}	102 ± 3.109	0.3495^{ns}
Avg. Egg Production /day	8.675 ± 0.629	$0.087^{4n}s$	9.875 ± 0.818	0.3495^{ns}
Egg weight (g)	43.35 ± 1.556	0.0872^{ns}	41.603 ± 1.432	0.0002^{***}
Mortality (%)	0.05 ± 0.1	0.0519^{ns}	0.05 ± 0.1	0.0519^{ns}
FCE	2.3175 ± 0.238	0.7015^{ns}	2.45 ± 0.056	0.2419^{ns}
TMCT				
	For Group Fayoumi		For Group Black Australorp	
Factors	Comparative groups	Level of significance	Comparative groups	Level of significance
Egg weight (g)	-	-	Control vs A1B	**
	-	-	Control vs A2B	***
	-	-	Control vs A3B	***

Saccharomyces cerevisiae on egg quality parameters determined through one way ANOVA and TMCT are summarized in Table 4. Significant improvement in egg quality for each treatment was found in yolk weight, yolk ratio, egg shell ratio, albumin weight, egg shell weight, and Haugh unit (shown in Fig. 2).

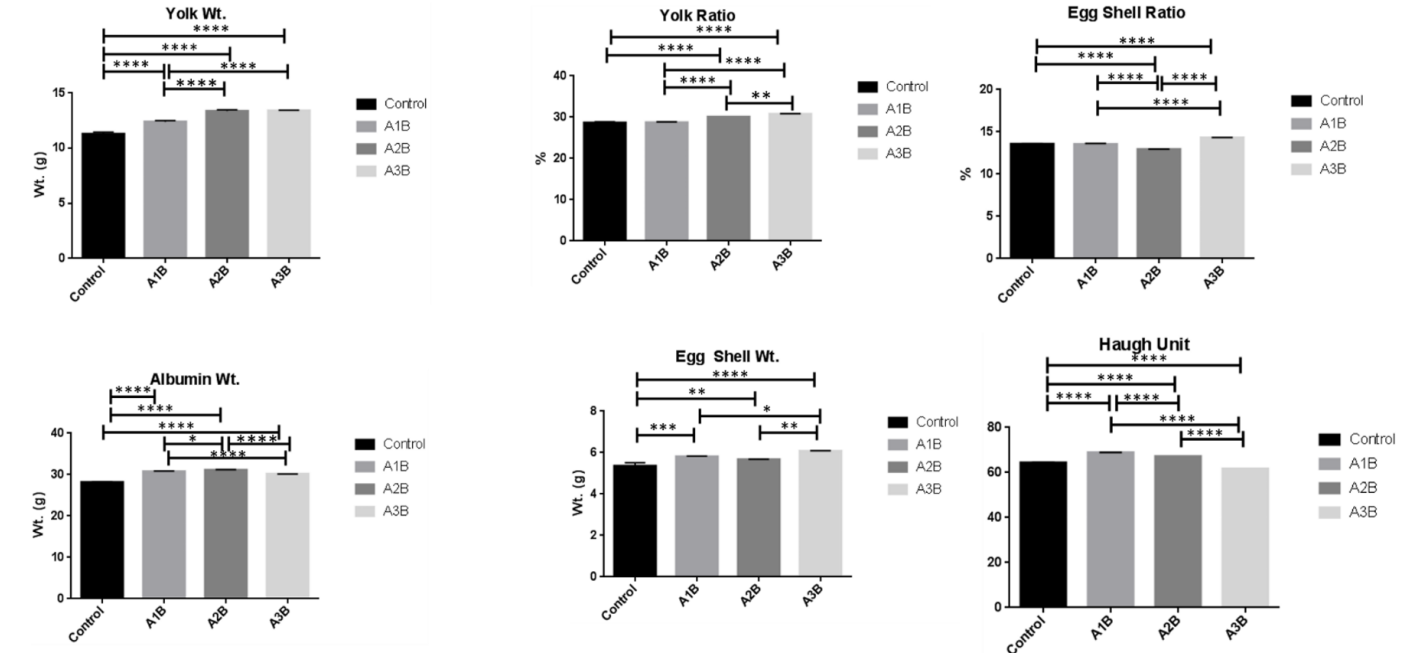

Figure 2 – Significant egg quality parameters are shown for Black Australorp birds.

Production Performance (Fayoumi)

No significant change was found in any parameter of production performance (Table 3).

Egg Quality (Fayoumi)

Only Haugh unit showed significant improvement in all treatment groups compared to the control group (Fig. 3).

Immune Responses

The geometric mean titers (GMT) obtained after the HI assay of treated birds was higher than that

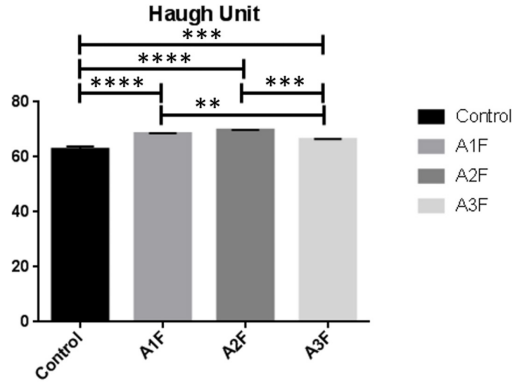

Figure 3 – Significant egg quality parameter is shown for Fayoumi birds.

Table 4 – Effect of *Saccharomyces cerevisiae* on egg quality parameters.

One way ANOVA				
	For Group Fayoumi		For Group Black Australorp	
Factors	Mean ± SD	*p*-value	Mean ± SD	*p*-value
Egg Weight(g)	42.258 ± 0.518	0.4830^{ns}	42.685 ± 2.231	<0.0001****
Yolk Weight (g)	12.913 ± 0.369	0.8346^{ns}	12.625 ± 1.001	<0.0001****
Yolk Ratio%	30.563 ± 0.824	0.1323^{ns}	29.566 ± 1.053	<0.0001****
Yolk Color	8.32 ± 0.5	0.8652^{ns}	8.800 ± 0.5	0.5523^{ns}
Albumin weight (g)	29.104 ± 0.524	0.4065^{ns}	30.043 ± 1.33	<0.0001****
Albumin Ratio %	69.188 ± 0.824	0.4122^{ns}	70.453 ± 1.053	0.1410^{ns}
Egg White Height (mm)	5.420 ± 0.05	0.8142^{ns}	5.394 ± 0.052	0.9906^{ns}
Egg Shape index	1.133 ± 0.041	0.9666^{ns}	1.183 ± 0.056	0.6407^{ns}
Egg Shell Weight(g)	5.727 ± 0.229	0.9979^{ns}	5.728 ± 0.301	<0.0001****
Egg Shell Ratio %	13.583 ± 0.012	0.9738^{ns}	13.583 ± 6.094	<0.0001****
Shell Thickness (mm)	0.308 ± 0.0465	0.9757^{ns}	0.308 ± 0.565	0.7454^{ns}
Haugh unit	65.485 ± 3.041	<0.0001****	65.485 ± 3.175	<0.0001****
TMCT				
	For Group Fayoumi		For Group Black Australorp	
Factors	Comparative groups	Level of significance	Comparative groups	Level of significance
Egg Weight(g)	-	-	Control vs A1B	****
	-	-	Control vs A2B	****
	-	-	Control vs A3B	****
	-	-	A1B vs A2B	****
	-	-	A1B vs A3B	****
	-	-	A2B vs A3B	****
Yolk Weight (g)	-	-	Control vs A1B	****
	-	-	Control vs A2B	****
	-	-	Control vs A3B	****
	-	-	A1B vs A2B	****
	-	-	A1B vs A3B	****
Yolk Ratio%	-	-	Control vs A2B	****
	-	-	Control vs A3B	****
	-	-	A1B vs A2B	****
	-	-	A1B vs A3B	****
	-	-	A2B vs A3B	**
Albumin weight (g)	-	-	Control vs A2B	****
	-	-	Control vs A3B	****
	-	-	A1B vs A2B	*
	-	-	A1B vs A3B	****
	-	-	A2B vs A3B	****
Egg Shell Weight(g)	-	-	Control vs A1B	***
	-	-	Control vs A2B	**
	-	-	Control vs A3B	****
	-	-	A1B vs A3B	*
	-	-	A2B vs A3B	**
Egg Shell Ratio %	-	-	Control vs A2B	****
	-	-	Control vs A3B	****
	-	-	A1B vs A2B	****
	-	-	A1B vs A3B	****
	-	-	A2B vs A3B	****
Haugh unit	Control vs A1F	****	Control vs A1B	****
	Control vs A2F	****	Control vs A2B	****
	Control vs A3F	***	Control vs A3B	****
	A1B vs A3B	**	A1B vs A2B	****
	A2B vs A3B	***	A1B vs A3B	****
			A2B vs A3B	****

of the control group (Table 5). Probiotic inclusion in diet resulted in an increased antibody titer against ND as compared to the control. Similarly, titers obtained in response to ELISA performed for IB was also significantly higher in treatment groups (Table 5).

Table 5 – Effect of *Saccharomyces cerevisiae* on antibody titers against New Castle Disease and Infectious Bronchitis Disease.

	Parameters	Groups							
		A1B	A2B	0 A3B	CB	A1F	A2F	A3F	CF
ELISA (IBV Titer)	O. D	0.523	0.449	0.637	0.189	0.449	0.491	0.637	0.086
	Mean	0.523	0.449	0.637	0.189	0.449	0.491	0.637	0.086
	S/P	5.16	3.48	4.14	1.25	3.49	3.86	5.17	0.24
	Titer	13717	8938	10799	2922	8938	9990	8999	484
	Log2	14	13	13	12	13	13	13	9
	Result	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
H.I (ND Titer)	H.I Test reading (GMT log-2)	10	8	9	7	9	9	9	8

DISCUSSION

No considerable effects on body weight gain have been reported by using probiotics for layers Yalçın *et al*. (2012); Hassanein & Soliman (2010). Likewise, no significant body weight gain was obvious during this study. In contrast, Shareef & Al-Dabbagh (2009); Fasina & Thanissery (2011); Fathi *et al*. (2012) have shown positive weight gain due to the supplementation of probiotics in broilers, which may involve certain other factors including difference in strains and species of animals used.

Maximum weight of egg ($p<0.05$) was seen in Black Australorp hens which were given 0.5 gm of 44.5 gm during the experiment. Our findings were consistent with other studies Ayanvale *et al.* (2006); Yalçin *et al*. (2008); Yalçın *et al*. (2015); Zhong *et al*. (2016), but differed from Nursoy *et al*. (2004); Asli *et al*. (2007); Hassanein & Soliman (2010); Gül *et al*. (2013); Sacakli *et al*. (2013).

Shape index, albumin & yolk weight, and shell weight were not statistically different. The results were consistent with the studies of Asli *et al*. (2007); Hassanein & Soliman (2010); Yalçın *et al*. (2012); Yalçın *et al*. (2014); Yalçın *et al*. (2015). The highest yolk weight and percentage was found in the group A3B fed 1.5 gm of yeast. The lowest albumen percentage was also observed in the same group. The maximum Haugh Unit (HU) was found in the Fayoumi breed supplemented with 1.0 mg of probiotic per kg. Similarly, earlier studies Ayanwale *et al*. (2006); Yousefi & Karkoodi (2007); Asli *et al*. (2007); Zhong *et al*. (2016) reported positive effects on birds by supplementing yeast.

The current study revealed that resistance against diseases improved in hens, but no significant effect on body weight was observed due to probiotic supplementation. ND and IB titers were high in all treatment groups when compared to control, which may be explained by an increase in availability of serum immunoglobulin. Systemic antibodies in response to various antigens may get modulated due to Probiotics Huang *et al*. (2004). In another study, a significant increase in serum antibodies was found in response to sheep red blood cells (SRBC) by Haghighi *et al*. (2006) when compared to control ones. Kabir *et al*. (2004) assessed the activity of probiotics on broiler's immune response and reported a considerably high antibody response in trial birds as compared to the controls. Cheng *et al*. (2004) suggested that probiotic feeding enhanced some cell mediated immune responses in broilers by modifying macrophage activity. Our results were consistent with Shoeib *et al*. (1997), who found an increase in the number of follicles in the Bursa of Fabricious in a probiotic treated group, ultimately resulting in high medullar plasma cell reaction.

CONCLUSION

Experimental findings concluded that *Saccharomyces cerevisiae* supplementation has minimal effect on weight gain and feed intake, but has more pronounced effects on egg production in Black Austarlorp and Fayoumi layers. Egg Quality parameters were not affected by *Saccharomyces cerevisiae* inclusion in feed.

ACKNOWLEDGEMENTS

The kind supervision and valuable comments of Dr. Abdul Rehman, Director Poultry Research Institute, Rawalpindi, Pakistan during the trial is highly acknowledged.

CONFLICT OF INTEREST

No potential conflict of interest was reported by authors.

REFERENCES

Abdulrahim SM, Haddadin MSY, Hashlamoun EAR, Robinson RK. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. British Poultry Science 1996;37:341-6.

Apata DF. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. Journal Science Food Agriculture 2008;88:1253-8.

Asli MM, Hosseini SA, Lotfollahian H, Shariatmadari F. Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. International Journal of Poultry Science 2007;6:895-900.

Ayanwale BA, Kpe M, Ayanwale VA. The effect of supplementing *Saccharomyces cerevisiae* in the diets on egg laying and egg quality characteristics of pullets. Journal of Poultry Science 2006;5:759-63.

Balevi T, Ucan US, Coşun B, Kurtoğu V, Cetingül IS. Effect of dietary probiotic on performance and humoral immune response in layer hens. British Poultry Science 2001;42:456-61.

Cheng YH, Lee DN, Wen CM, Weng CF. Effects of β-glucan supplementation on lymphocyte proliferation, macrophage chemotaxis and specific immune responses in broilers. Asian-Australasian Journal Animal Science 2004;17:1145-9.

Ehrmann MA, Kurzak P, Bauer J, Vogel RF. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. Journal Applied Microbiology 2002;92:966-75.

Fasina YO, Thanissery RR. Comparative efficacy of a yeast product and bacitracin methylene disalicylate in enhancing early growth and intestinal maturation in broiler chicks from breeder hens of different ages. Poultry Science 2011;90(5):1067-73.

Fathi MM, Al-Mansour S, Al-Homidan A, Al-Khalaf A, Al-Damegh M. Effect of yeast culture supplementation on carcass yield and humoral immune response of broiler chicks. Veterinary World 2012;5:651-7.

Forte C, Moscati L, Acuti G, Mugnai C, Franciosini MP, Costarelli S, et al. Effects of dietary *Lactobacillus acidophilus* and *Bacillus subtilis* on laying performance, egg quality, blood biochemistry and immune response of organic laying hens. Journal of Animal Physiology and Animal Nutrition 2016;100:977-87.

Haghighi HR, Gong J, Gyles CL, Hayes MA, Zhou H, Sanei B, et al. Probiotics stimulate production of natural antibodies in chickens. Clinical and Vaccine Immunology 2006;1:975-80.

Hassanein SM, Soliman NK. Effect of probiotic (*Saccharomyces cerevisiae*) adding to diets on intestinal microflora and performance of Hy-Line layers hens. Journal of American Science 2010;6:159-69.

Huang Z, Elankumaran S, Yunus AS, Samal SK. A recombinant Newcastle disease virus (NDV) expressing VP2 protein of infectious bursal disease virus (IBDV) protects against NDV and IBDV. Journal of Virology 2004;78:10054-63.

Jin LZ, Ho YW, Abdullah N, Jalaludin S. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus cultures*. Poultry Science 1998;77:1259-65.

Khan SH, Yousaf B, Mian AA, Rehman A, Farooq MS. Assessing the effect of administering different probiotics in drinking water supplement on broiler performance, blood biochemistry and immune response. Journal Applied Animal Research 2011;39:418-28.

Kumprecht I, Zobac P. The effect of probiotic preparations containing *Saccharomyces cerevisiae* and *Enterococcus faecium* in diets with different levels of beta-vitamins on chicken broiler performance. Czech Journal of Animal Science-UZPI 1998;43(2):63-70.

Leeson S. Future considerations in poultry nutrition. Poultry Science 2012;91:1281-5.

Martin SA, Nisbet DJ, Dean RG. Influence of a commercial yeast supplement on the in vitro ruminal fermentation. Nutrition Reports International 1989;40:395-401.

Midilli M, Alp M, Kocabach N, Muglah OH, Turan N, Yilmaz H, et al. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. South African Journal Animal Science 2008;38:21-27.

Mikulski D1, Jankowski J, Naczmanski J, Mikulska M, Demey V. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poultry Science 2012;91:2691-700.

Nayebpor M, Farhoman, P, Hashemi A. Effects of different levels of direct fed microbial (Primalac) on growth performance and humoral immune response in broiler chickens. Journal of Animal and Veterinary Advances 2007;6:1308-13.

Netherwood T, Gilbert HJ, Parker DS, O'donnell AG. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Applied and Environmental Microbiology 1999;65:5134-8.

Nursoy H, Kaplan O, Oguz MN, Yilmaz O. Effects of varying levels of live yeast culture on yield and some parameters in laying hen diets. Indian Veterinary Journal 2004;81:59-62.

Patterson JA, Burkholder KM. Application of prebiotics and probiotics in poultry production. Poultry Science 2003;82:627-31.

Saçaklı P, Köksal BH, Ergün A, Özsoy B. Effects of using inactivated brewer's yeast (*Saccharomyces cereviciae*) in diets as a source of vitamin and mineral in performance of broilers. Veterinarija ir Zootechnika 2011;61:53-60.

Salianeh N, Shirzad MR, Seifi S. Performance and antibody response of broiler chickens fed diets containing probiotic and prebiotic. Journal of Applied Animal Research 2011;39:65-7.

Shareef AM, Al-Dabbagh ASA. Effect of probiotic (*Saccharomyces cerevisiae)* on performance of broiler chicks. Iraqi Journal of Veterinary Sciences 2009;23:23-9.

Thayer SG. Serologic procedures. In: Swayne DE, editor. A laboratory manual for the isolation and identification of avian pathogens. 4th ed. Philadelphia: American Association of Avian Pathologists; 1998. p.256-8.

Yalçin S, Özsoy B, Erol H, Yalçin S. Yeast culture supplementation to laying hen diets containing soybean meal or sunflower seed meal and its effect on performance, egg quality traits, and blood chemistry. Journal Applied Poultry Research 2008;17:229-36.

Yalçın S, Uzunoğlu K, Duyum HM, Eltan Ö. Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) and black cumin seed (*Nigella sativa* L.) on performance, egg traits, some blood characteristics and antibody production of laying hens. Livestock Science2012;145:13-20.

Yalcin S, Yalcin S, ŞAHİN A. Effects of dietary inactive yeast and live yeast on performance, egg quality traits, some blood parameters and antibody production to SRBC of laying hens. Kafkas Universitesi Veteriner Fakultesi Dergisi 2015;21:345-50.

Yalçın S, Yalçın S, Onbaşılar İ, Eser H, Şahin A. Effects of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens. Veteriner Fakultesi Dergisi 2014;61:289-94.

Yeo J, Kim KI. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Science1997;76:381-385.

Yousefi M, Karkoodi K. Effect of probiotic Thepax® and *Saccharomyces cerevisiae* supplementation on performance and egg quality of laying hens. International Journal of Poultry Science 2007;6:52-54.

Zhang ZF, Kim IH. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poultry Science 2014;93:364-70.

Zhong S, Liu H, Zhang H, Han T, Jia H, Xie Y. Effects of *Kluyveromyces marxianus* isolated from tibetan mushrooms on the plasma lipids, egg cholesterol level, egg quality and intestinal health of laying hens. Brazilian Journal of Poultry Science 2016;18:261-8.